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PREPUPAL CHANGES IN THE VENTRICULAR EPITHELIUM OF THE EUROPEAN CORN BORER, PYRAUSTA NUBILALIS (HUBN.)[1]

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University of Wisconsin

An investigation of the digestive epithelium of the European corn borer during the winter diapause was carried out in conjunction with nutrition and food habit studies of the larvae of this insect. Histological changes associated with metamorphosis were observed at the onset of diapause, and the study became one of tracing the prepupal changes in the epithelium of the mid-gut. The European corn borer passes the winter as a mature larva in diapause. The larva pupates in the spring without further feeding. The use of the overwintering generation of larvae for studies of prepupal changes has an advantage in that the rate of the developmental changes that occur is greatly retarded by the low environmental temperatures. The several phases of metamorphosis are therefore more distinctly separate than is the case with the generation which matures and pupates during the summer. The observations reported here were repeated in studies of the histological changes associated with metamorphosis in larvae which pupated during the summer, and the same developmental changes were found to occur.

Considerable literature is available on the histology and cytology of the mid-gut of lepidopterous larvae. However, relatively little has been published on the histological and cytological changes in the mid-gut during metamorphosis. In a classic work on this subject, Deegener (1908) described metamorphosis of the digestive tract of *Malacosoma castrensis* in great detail. Ito (1920) studied the metamorphosis of the digestive tract of *Bombyx mori*. As far as the mid-gut

[1]This investigation was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. Approved for publication by the director of the Wisconsin Agricultural Experiment Station.

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was concerned, Ito's observations were in complete accord with those of Deegener. Henson (1930) published on the early mid-gut development of *Vanessa urticae* and the late (fifth instar) mid-gut development of *Pieris brassicae*. Although Henson did not carry the study unto the pupal stages, his observations on the prepupal stages in *Pieris* were in agreement with those of Deegener.

The only paper known to the writers which is devoted to the anatomy of the mid-gut of the European corn borer is that of Buchmann (1928), in which the development and secretory activity of the epithelial cells was traced from the first to the last (fifth) instar, but in which no account of changes associated with metamorphosis was given.

MATERIAL AND METHODS

The larvae utilized in this study were collected from the field at regular intervals from early fall until pupation was nearly complete in the spring. Immediately after collection, the larvae were fixed in Bouin's fixative at 65° C. The warm Bouin's was injected into the hemocoel, after which the larvae were dropped into the warm fixative. The fixative was then brought to room temperature. After about twenty-four hours in this solution, the digestive tracts were removed by dissection and rinsed in 70% ethyl alcohol until the yellow color of the fixative was no longer evident. They were then dehydrated by the n-butyl alcohol method of Stiles (1934) and imbedded in paraffin. Serial sections were stained with Heidenhain's iron-haematoxylin and counterstained with Fast Green FCF.

In the case of the histochemical methods employed, the above procedure was modified as necessary. For the detection of fat, the larvae were fixed in cold calcium-formol according to the technique of Baker (1946). The tissue was sectioned in a frozen state and stained with Sudan Black. For the detection of glycogen, the larvae were fixed in a solution consisting of nine parts of a saturated alcoholic solution of picric acid and one part of formalin. The paraffin sections were stained with Best's carmine by the method given by Conn and Darrow (1946). Uric acid in the tissues was detected by the technique of Hollande (1931) in which the tissue is fixed in the dark with a solution of silver nitrate and neutral formalin. Paraffin sections were either left unstained or were stained lightly with haemalum and eosin.

RESULTS

The epithelium of the ventriculus of the immature last instar larvae of the European corn borer appeared to be quite typical of the condition reported in the literature for lepidopterous larvae in general and by Buchmann (1928) for *Pyrausta*. Figure 1 is a photomicrograph of the ventricular epithelium of a feeding larva. The mucosa consists of tall columnar cells with prominent striated borders, and numerous goblet cells. Regenerative cells are scattered singly or in small groups along the distinct basement membrane. Although it is not shown in figure 1, a peritrophic membrane was always present in feeding larvae.

At larval maturity, feeding ceased and the gut tract was evacuated of ingesta and peritrophic membrane. At this time, or slightly earlier,

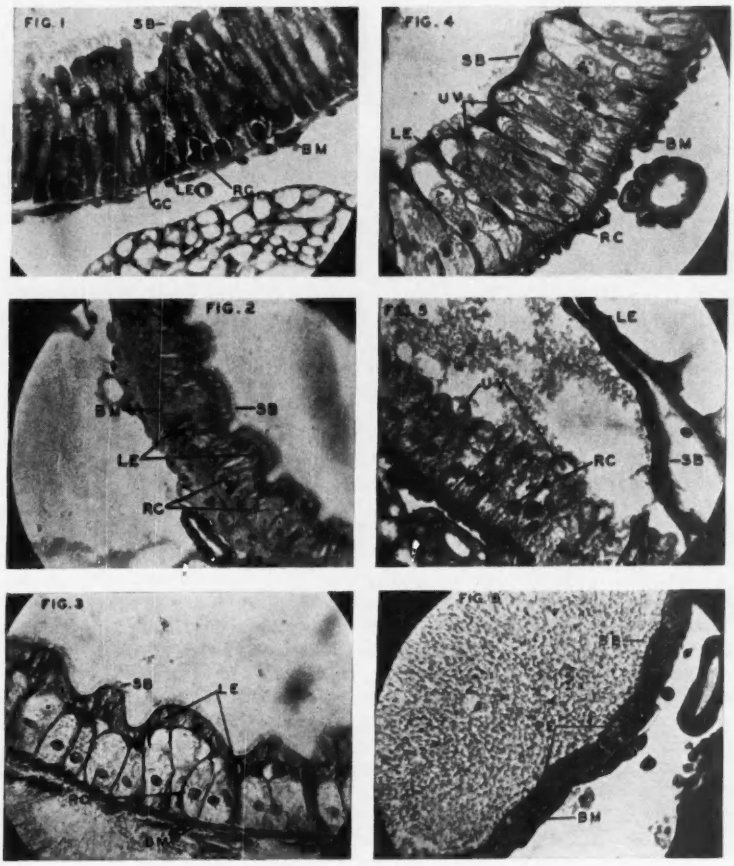

FIG. 1. Mucosa of the ventriculus of an immature last instar European corn borer larva. FIG. 2. Mucosa of the ventriculus of a mature last instar European corn borer larva shortly after feeding ceased. FIG. 3. Mid-winter mucosa of the ventriculus of a European corn borer larva. FIG. 4. Early prepupal mucosa of the ventriculus of a European corn borer. FIG. 5. Ventricular mucosa of late prepupa or early pupa of the European corn borer. FIG. 6. Early pupal ventricular epithelium of the European corn borer.

ABBREVIATIONS USED IN FIGURES

BM—Basement membrane.
GC—Goblet cell.
LE—Larval digestive epithelium.
PE—Pupal ventricular epithelium.
RC—Regenerative cell.
SB—Striated border.
UV—Urate vacuole.

the regenerative cells began to enlarge and became very prominent. No mitotic figures were observed, and the apparent increase in regenerative cells was interpreted as being solely in cell size, rather than in both size and number. Figure 2 shows the early stages of this process. It can be observed in this figure that the enlarged regenerative cells became round and that their cytoplasm did not stain as deeply as did the cytoplasm of the columnar cells. Goblet cells were no longer detectable, but the striated border remained visible. The larval digestive epithelium showed no evidence of secretory activity at this or any subsequent stage. The basement membrane was still present, although the "connective tissue" observed by Deegener (1908) appeared to have been formed. The basement membrane did not disappear as observed by Deegener in *Malacosoma*. That the enlarged cells were the original regenerative cells was indicated by two observations: (1) the complete disappearance of the small basal cells as the layer of enlarged cells became more prominent, and (2) the appearance of all intergradations between unmodified regenerative cells and the large cells during the early stages of the process.

The enlargement of the regenerative cells continued until a continuous layer of the large, lightly staining rounded cells was formed. Figure 3 shows a typical section at this stage. The striated border of the larval digestive epithelium remained prominent. The nuclei of the latter cells began to show signs of pyknosis in some areas. The larval digestive cells are shown to be much crowded and reduced, but it was observed that they retained their connections to the basement membrane. This is shown in figure 3 by the thin strands of cytoplasm extending between the regenerative cells and to the basement membrane. This was found to be the typical stage in which the larvae passed the winter. No further changes were detected until activity was resumed in the spring.

The observations made on the larvae during the mid-winter stage are not in strict agreement with those of the earlier workers. Ito (1920) reported the larval digestive epithelium of *Bombyx* to have lost its striated border at about this point. However, the present writers found that in the corn borer larva the striated border was maintained until well after the larval digestive epithelium was sloughed off into the lumen (see figure 5). Deegener (1908) with *Malacosoma* and Ito (1920) with *Bombyx* described the early appearance of an extensive irregular vacuole layer between the larval digestive epithelium and the layer of enlarged regenerative cells. Deegener considered the vacuoles as being from both cell layers, but Ito implied that they arose from the basal portions of the digestive epithelium. Deegener observed that the vacuolar layer was probably the only factor holding the larval digestive epithelium and the regenerative cells together, and that when the vacuoles later ruptured, the two cell layers separated. In the corn borer larvae vacuoles of this type were not observed, and the digestive epithelial cells remained attached to the basement membrane until they were cast off into the gut lumen. Both Deegener and Ito observed a dissolution and reformation of the basement membrane; whereas, the present writers did not observe this phenomena in the European corn borer.

Histochemical tests for stored fat and glycogen in the regenerative cells during the winter stage were inconclusive. Fat droplets were detected in the cells, but not in quantities sufficient to indicate positively that fat was being stored. Glycogen tests were largely negative, although a little glycogen was found in some of the cells.

Further histological changes in the mid-gut epithelium became apparent during the early spring. The regenerative cells lost their rounded or polyhedral form and increased in height to become tall columnar cells. Shortly after the development of the columnar form, a vacuole developed in the apical region of each cell. Figure 4 depicts the situation at this stage. The deeply staining granular inclusions of the apical vacuoles were identified histochemically as uric acid or urates. These apical urate vacuoles were referred to as extraneous nuclei by Deegener (1908).

As development proceeded, the vacuoles became larger and more distinctly apical, until they projected from the distal ends of the cells as prominent bulb-like formations. The vacuoles began to burst and the larval digestive epithelium began to slough off to form the so-called yellow body. In many regions of the gut, the larval epithelium was cast off before the vacuoles burst. Figure 5 shows the beginning of the vacuole rupturing stage. The granular material in the gut lumen was not evident until the urate vacuoles began to rupture. The dissolution of the yellow body was probably enzymatic, since no invasion by phagocytes was observed. It is very likely that any enzymes present were formed by the regenerative cells, although probably not in the urate vacuoles. At the stage shown in figure 5, the larvae were in the characteristic prepupal posture.

After the beginning of the vacuolar disintegration, the nuclei of the regenerative cells moved toward the cell bases. The cytoplasm of the cells continued to pour out into the lumen, until the cells were only about one-fourth of their original height. In some areas the entire cell layer appeared to be lost, but in most regions the nuclei and a thin cytoplasmic layer were not lost, and the cells began to repair. The cells, now of a low cuboidal type, reformed their distal cell walls and developed a striated border. Figure 6 shows a typical section at this stage. It was observed that the previously convoluted basement membrane became straightened out, as a result of which the cross-section diameter of the gut was increased considerably. This increase in diameter probably was due to the bulk of the granular mass secreted into the lumen by the cells. At the time of pupation the mid-gut epithelium was in about the state of development shown in figure 6. From this low cuboidal pupal epithelium, the imaginal epithelium is presumed to arise.

SUMMARY

The prepupal changes in the mid-gut epithelium of both the summer and the over-wintering generations of the European corn borer may be summarized as follows:

1. The regenerative cells of the larval ventricular mucosa enlarge to form a continuous layer.

2. The regenerative cells become columnar and develop apical vacuoles containing uric acid or urates.

3. The larval digestive epithelium is cast off into the gut lumen.

4. The distal portions of the regenerative cells disintegrate into the lumen.

5. The nuclei and basal portions of the regenerative cells form a low cuboidal pupal epithelium with a distinct striated border.

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THE SOCIETY OF SYSTEMATIC ZOOLOGY

The Society of Systematic Zoology, formed in 1948, is the result of recognition of the need for a means of bringing taxonomists together to represent their interests among zoologists in general, and to improve taxonomic practice and training through united action. That taxonomists see the advantage to such association is attested by the fact that more than 700 have joined the Society during its three years of existence. At the present rate of growth the goal of 1000 members should easily be met in 1951.

The history and projects of the Society have been outlined in its News Letter No. 2, published in March 1950. Its logical field of activity includes all the systematic fields of zoology and paleontology, and all persons interested in any way in systematics or any of the related fields will be welcomed to membership.

Among the projects in preparation or under consideration by the Society are an advanced textbook of the principles of systematics, a journal of the principles of systematic zoology, studies of the current practices and needs of teaching in taxonomy, improving publication outlets for systematic papers, preparation of faunal handbooks to fill gaps in the present literature, cooperation with the Zoological Record, standardization of practices and terminologies, sponsoring of annual meetings for personal contact and presentation of papers, an identification service, representation on the American Association for the Advancement of Science, the American Institute of Biological Sciences, the National Research Council, and similar organizations, and sponsoring group opinion on such problems as microfilm publication of new names, the copyright of the publications of the International Commission on Zoological Nomenclature, and the international nomenclature situation.

Entomologists were not among the groups most widely reached by the early announcements. Their full participation, however, is essential to full influence of the Society and they are cordially invited to join. Address inquiries to Dr. C. Clayton Hoff, (Chairman of the Membership Committee), University of New Mexico, Albuquerque, New Mexico.

BRONTISPA YOSHINOI BARBER, A DESCRIPTION OF ADULT AND IMMATURE STAGES[1]

(Coleoptera: Hispidae)

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University of California

The coconut pest from the Palau Islands, *Brontispa yoshinoi* Barber, was first recorded by Chujo (1937) who misidentified it as *Planispa chalybeipennis* (Zacher). Later Esaki (1940) recognized this Palauan insect as an undescribed blue species of the genus *Planispa*.

Apparently the same species was observed by Oakley (1946) who states: "? *Brontispa* sp., a blue colored hispine beetle feeding on leaflets in the crown was taken from coconuts on both Babelthuap and Peleliu. Its type of injury to coconuts was similar to that of *Brontispa mariana* Spaeth in Truk and the Marianas, in that it attacks newly exposed leaf tissue and leaves a frond soon after it is fully spread. Attacks of the pest in the Babelthuap locality surveyed seemed light and insignificant, but perhaps 10 per cent of the palms in a Peleliu planting showed some sign of infestation with an occasional palm being seriously affected."

The junior author conducted a survey in 1948 for the Pacific Science Board of the National Research Council and found *B. yoshinoi* to be generally distributed over southern Babelthuap, Koror, Arakabasan, and Peleliu Islands. The most severe damage to palms was noted on Peleliu and the infestation was greater than that described by Oakley (1946). The beetle apparently has habits nearly identical with *B. mariana*, a species which has not been found in the Palau group although it occurs throughout the Caroline islands lying to the north and east. In the Palau Islands its ecological niche is nicely filled by *B. yoshinoi*.

Brontispa yoshinoi Barber

(Figs. 1–17)

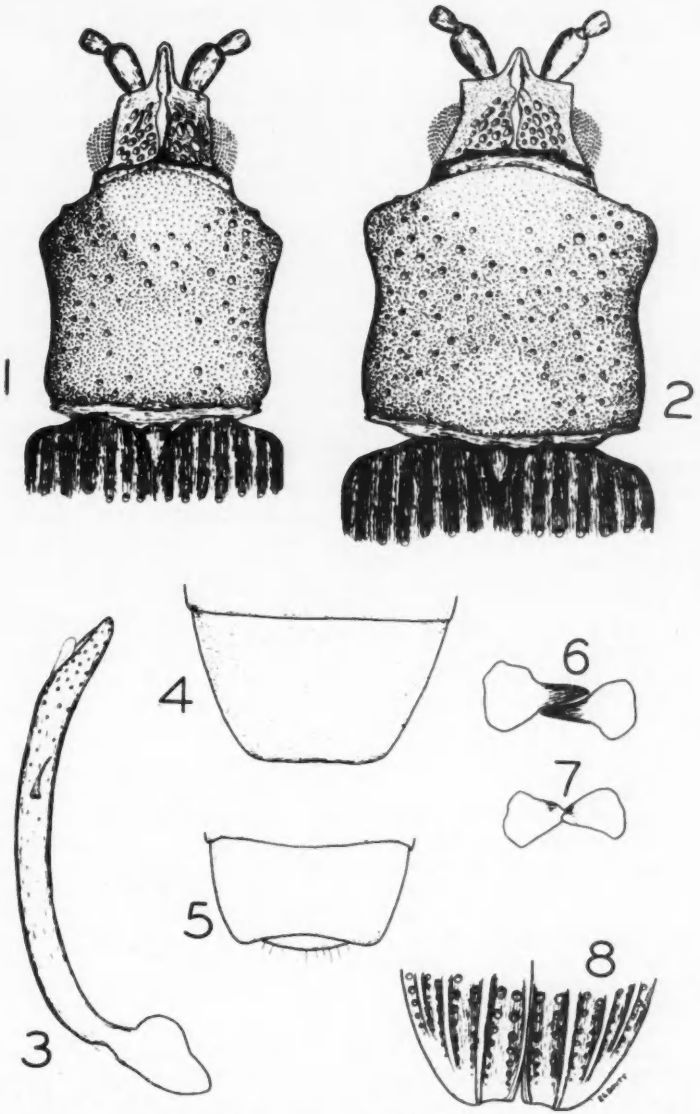
Planispa chalybeipennis, Chujo (not Zacher, 1915), 1937, Trans. Nat. Hist. Soc. Formosa, 27: 227, figs. 5, 6, 7, 9. (Misidentification.)

Brontispa chalybeipennis, Lepesme (not Zacher, 1915), 1947, Les Insectes des Palmiers, p. 545. (In part misidentified.)

Male.—Form elongate, four times longer than wide, subparallel sided, depressed, shining; head, pronotum, scutellum piceous; elytra deep metallic blue, extreme apices brown; abdomen fuscous. *Head* (fig. 1) above as long as wide including anterior projection, from beneath 1.6 times longer than wide, nearly half the width of pronotum; elevated interocular area wider than long (36:27) excluding anterior process, width greatest at base, tapering slightly toward anterolateral angles,

[1]The following paper originally described the species as new. The appearance of a description by H. S. Barber of apparently the same species in the Jour. Wash. Acad. Sci., 40: 245–247, 1950, made it necessary to change this designation to: *Brontispa yoshinoi* Barber.

the latter feebly dilated with pointed apices projecting laterally, anterior margin of interocular area feebly obliquely arcuate with elongate process arising from middle and extending anteriorly between basal antennal segments, interocular surface with distinct, deep, median longitudinal sulcus sinuately outlined, widest at anterior margin, constricting posteriorly then expanding at middle and finally constricting so sides of sulcus nearly contiguous at base, elevated surface adjacent to sulcus coarsely, irregularly punctate, approaching rugosity, finely rugulose laterally and anteriorly; inter-antennal process narrow, about two and one-half times as long as width at base, nearly as long as first antennal segment, sides subparallel, feebly accuminate, narrowing anteriorly, terminating into broadly rounded apex, margins entire, upper surface with deep sulcus continuing from interocular structure, widest at base, narrowing gradually toward apex, outlined by vertical side margins, surface of sulcus very finely reticulate, deepest anteriorly; interocular area (clypeus) beneath slightly elevated, with surface rather coarsely, shallowly punctate and microscopically reticulate, clothed with abundant fine erect brownish pubescence confined to elevated plateau; antennae slightly longer than head and thorax combined, distinctly shorter than one-third length of body, dark brown above, paler beneath, first segment longest and broadest, nearly twice as long as broad, second shortest, third slightly longer, first segment as long as second and third combined, four to six subequal in length, seventh longest of flageller segments, four distal segments feebly compressed laterally, clothed with very short appressed pile confined to dorso-ventral surfaces (appearing striped); mandibles blunt, bidentate, incisor tips slightly overlapping (fig. 7). *Pronotum* (fig. 1) very slightly longer than wide (45:42), length equal to width of elytra at base; sides nearly parallel from base to cephalic third thence expanded, forming rounded more or less projecting anterolateral angles, widest at this point, each posterolateral angle bearing carina-like tooth at margin projecting more or less laterally, lateral pronotal margins sharp, each inner angle of anterolateral projection bearing single small rounded tooth, anterior margin extending between latter teeth broadly rounded and convex; upper surface very sparsely and finely punctate, with large scattered conspicuous punctures becoming most numerous on lateral areas at front and fewer on posterior lateral areas, absent on anterior convex surface, rather sparse on disc; inflexed sides finely reticulate with few very large, deep punctures restricted more or less to anterior half; prosternal disc glabrous, sparsely finely punctate; mesosternum with somewhat larger punctures than on preceding structure and with several shallow feeble longitudinal impressions at middle; metasternal sculpturing similar to prosternal disc. *Scutellum* triangular with sides slightly curved outwardly, surface microscopically reticulate, base depressed. *Elytra* three times as long as wide; base bisinuate, wider than base of pronotum; humeri broadly rounded, exposed; sides subparallel, feebly sinuate being slightly constricted near basal third, thence slightly divergent posteriorly and very feebly arcuate reaching widest point just behind middle, then gradually and arcuately convergent to apex in apical fourth, margins sharp; apices (fig. 8) truncate, truncature very feebly inwardly oblique toward the very slightly projecting subspinose sutural angles, limited



Brontispa yoshinoi Barber, morphological characters of adults. FIG. 1. Dorsal view of ♂ head and pronotum. FIG. 2. Dorsal view of ♀ head and pronotum. FIG. 3. Lateral view of ♂ genitalia. FIG. 4. Ventral view of ♀ fifth abdominal sternite. FIG. 5. Ventral view of ♂ fifth abdominal sternite and of pygidial apex. FIG. 6. Mandibles of *Brontispa mariana* Spaeth. FIG. 7. Mandibles of *B. yoshinoi*. FIG. 8. Dorsal view of elytral apices showing costae and apical truncature.

laterally at junction of median longitudinal elytral costae with apical margin; surface glabrous, punctate-striate, punctures large, deep, with interstices on disc more or less equidistant, each elytron bearing nine rows, including scutellar and marginal rows on sub-basal area, postbasal area with eight rows excluding scutellar row, surface slightly behind middle with ten rows, sixth and seventh rows commencing near middle terminating near apical fifth, in apical area rows converging; apical area with distinct costae (fig. 8) appearing near origin of apical declivity, each elytron with a sutural, medial and two lateral costae, sutural and medial costae attaining apical margin. *Metathoracic wings* slightly longer than elytra when apices unfolded. *Legs* largely pale brown, femora distally darker; each trochanter bearing single more or less erect seta arising near pointed apex; each anterior femur armed with small, blunt carina-like tooth near base; each anterior tibia with oblique broad groove, near apex, margins of sulcus raised, appearing tooth-like, sulcus clothed with golden pile and accepts femoral tooth in tibial retraction. *Abdomen* in dissected paratopotypes with tergites sclerotized, surface finely reticulate with violaceous blue metallic luster; pygidium not exposed from above in natural repose, transverse, with apical margin broadly rounded, upper surface coarsely rather densely punctate, each large puncture with a rather long semi-erect seta projecting posteriorly, more abundant and stouter apically, thus apices of projecting setae visible from beneath; sternites glabrous, finely reticulate, particularly laterally, punctuation very fine and sparse; fifth sternite (fig. 5) as long as preceding sternite measured at middle, with apical margin broadly, arcuately emarginate, emargination limited laterally at postero-lateral angles, surface with somewhat more dense and coarse punctures than preceding sternites. *Genitalia* (fig. 3) from paratopotype.

Length, 7.9 mm; *width*, 1.8 mm.

Female.—Differs from male externally by the following characters: Form slightly larger throughout, not quite four times as long as wide; *head* (fig. 2) with interantennal projection shorter, only half the length of basal antennal segment, base wider, sides more rapidly acuminate terminating in very narrowly rounded apex, appearing pointed with little magnification; elevated *interocular area* much wider than long (40:25); *pronotum* (fig. 2) as long as wide; *elytra* not quite three times as long as wide, more dilated just behind middle; *abdomen* with fifth sternite (fig. 4) slightly longer than preceding sternite, apical margin apparently truncate but perceptibly very feebly broadly arcuately emarginate.

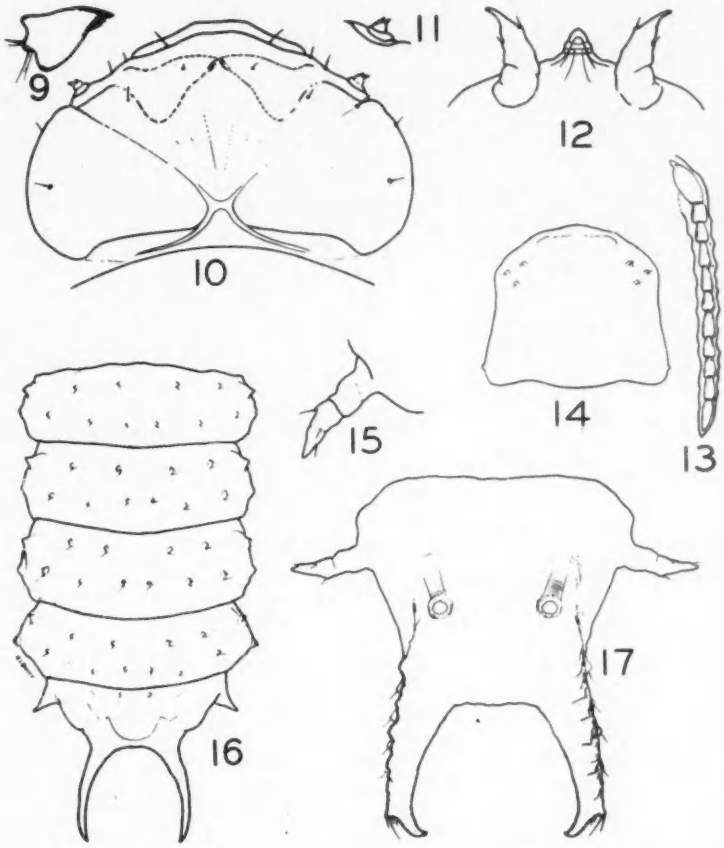
Length, 9.0 mm; *width*, 2.3 mm.

Variations.—Form rather constant, color of antennae, scutellum, legs and abdominal sternites varying from brown to piceous, at times pronotum fusco-piceous at sides; interantennal projection in males (viewed laterally) with apex varying from nearly parallel with the longitudinal axis to distinctly curved upward at about a 20° angle, apical margin varying from rather narrowly rounded to broadly rounded; pronotal punctuation variable, at times more dense on disc; clypeal pubescence absent in several paratopotypes (rubbed ?).

Length, ♂♂ 7.0-8.3 mm; ♀♀ 7.7-9.5 mm.

Width, ♂♂ 1.6–1.8 mm; ♀♀ 1.8–2.3 mm.

Sexual dimorphism.—As already described, the length of interantennal process and the shape of the apical margin of the fifth abdominal sternite are sexually dimorphic, and these particular characters were constant within the series studied. An additional secondary sexual character which appears to be rather constant is the elytral



Brontispa yoshinoi Barber, dorsal views of morphological characters of mature larva and pupa. FIG. 9. Left larval mandible. FIG. 10. Larval head capsule. FIG. 11. Larval antenna. FIG. 12. Apical region of pupal pronotum. FIG. 15. Lateral abdominal process of larva. FIG. 16. Last five abdominal tergites of pupa. FIG. 17. Eighth abdominal sternite of larva (tail-shovel).

length in relation to the width. The estimated standard error of the mean of the elytral lengths was obtained from random samples of ten specimens; in the females the average elytral length was 6.39 mm. ± 0.07 mm.; elytral length in the males: 5.42 mm. ± 0.08 mm; width of

male elytra: 1.69 mm. ± .03 mm; width of female elytra: 2.03 mm. ± .02 mm.

Egg.—Depressed, elliptical in outline, from twice as long as wide to two and one-half times as long as wide; ends broadly rounded; upper surface brown, darker at sides, slightly convex, distinctly coarsely, rather evenly reticulate, cells of reticulation either hexagonal or pentagonal, this sculpturing disappearing on ventral surface at middle, also paler and usually very feebly concave ventrally.

Length, 1.6–1.8 mm.

First instar larva.—Similar to mature larva but differing in having head comparatively larger in relation to body, clypeus more prominent; pronotum slightly wider and distinctly longer than either of remaining thoracic tergites; abdomen with lateral processes small, being inconspicuous knob-like structures and very slightly protruding from sides, these knob-like structures each bearing two or three rather long setae at apices; eighth tergite with largest lateral processes, obtuse apically, slightly constricted at base, tail-shovel not distinctly sclerotized, with spines obscure but with numerous lateral setae rather long and particularly abundant along lateral margins of prongs; prongs in outline distinctly more arcuately curved, each apex with acute, inwardly curved spine, space between prongs twice as broad as long at widest point.

Mature larva.—Body elongate, subparallel sided, very gradually narrowing cephalically, depressed; cuticle whitish to pale testaceous, with mandibles and tail-shovel darker. *Head capsule* (fig. 10) wider than long (70:42); basal margin, outlined by occipital suture, inwardly, obliquely arcuate becoming distinctly rather narrowly emarginate at middle forming short coronal suture; sides and anterior margin broadly rounded; cranial surface slightly convex, with a feeble median longitudinal impression narrowest near base of frons expanding anteriorly with widest point at anterior margin about width of clypeus, outlined at sides by apparent lines resembling sutures, but composed of minute tubercles arranged densely in lines, between lines smooth, remainder of surface rather densely tuberculate, tubercles flattened, rounded and slightly elevated (cleared specimens revealing post-frontal sutures originating from basal emargination, coronal suture, and running obliquely, cephalically to anterior margin just laterad of antennae), setae sparse, few confined to lateral and apical margins; antenna (fig. 11) three-segmented, basal segment largest, transverse, very short, second segment longer but much narrower than basal, slightly wider than long, third arising from outer side of apical flattened surface, another smaller segment arising inside and adjacent to third proper, narrowly rounded apically; ocelli arranged in transverse rows on anterior surface just laterad of antennal insertions, on each side with row of four ocelli arranged vertically with a smaller ocellus slightly posterior to ocelli row near middle; labrum barely visible from above; mandibles tridentate (fig. 9). *Thorax* with tergites distinctly broader than long, rectangular; ***pronotum*** slightly wider but very slightly shorter than head, anterolateral angles more acute than posterolateral angles, surface with sculpturing similar to head but intersegmental areas minutely spinulate, few hairs arising at lateral margins; ***mesonotum*** slightly wider but not as long as pronotum, lateral margins slightly bisinuate with shortly projecting setaceous knob-like processes at each side of transverse

median fold, surface microscopically spinulate; ***metanotum*** slightly wider but same length as preceding tergite, lateral margins, fold and surface similar to mesonotum; each intersegmental pleural area between pro- and mesothorax with a rather large conical laterally projecting process visible from above, opening into rounded spiracle, apically, trachea from spiracle enters mesothoracic cavity. ***Abdomen*** apparently eight segmented, first seven broader than long, nearly all same length but slightly increasing in width posteriorly with seventh tergite widest; eighth segment (fig. 17) (tail-shovel) longer than wide, forming terminal segment with two large posteriorly projecting prongs, antero-lateral margins angulate, sides parallel from angulation to lateral processes arising at basal-fifth, thence tapering to middle, from this narrowest width laterally diverging, forming lateral margins of prongs, elevated area and prongs somewhat sclerotized, elevated part beginning near basal-fourth, extending posteriorly at sides connecting with prongs, distance caudally from elevated base to truncate base between prongs nearly equal to length of prongs, concave surface with a large, rounded spiracle opening at each side near lateral margins of basal-fourth, distance between spiracles equal to width of truncate basal margin between prongs, feebly arcuate in outline at lateral margins, more so at inner margins, apices abruptly bent inwardly and upwardly with rather large but short spine at outer angles of inflection, extreme apices rather narrow and acute, dorsal lateral margins of elevated surfaces and prongs with seven or eight rather distinct spines without setae, ventrally the lateral margins of prongs bearing four spines equidistant from one another, each with curved seta arising at base, outer angle of prong apex with at least one seta, ventral surface of prongs each with four setae; abdominal surface microscopically spiculate, each tergite excepting eighth with transverse median fold devoid of spiculation; ***lateral processes*** (fig. 15) elongate, conical, projecting at right angles to lateral margin of each abdominal segment, from first and second segment arising from pleural area at middle, remaining processes arising posterior of middle excepting those on eighth, which arise anteriorly, each process two-thirds as long as length of tergite, broadest at base with sides acuminate, terminating in narrowly rounded apex, with a seta arising from feeble shelf-like process, at or near middle on dorsal surface and posterior side, another seta arising from anterior surface near apex, at times with a ventral subapical seta, surface rather densely spinulate (microscopically flattened spines); ***spiracles*** conspicuous, present on each segment, slightly projecting, nearly twice as wide as depth excepting on eighth, situated directly above lateral processes on first abdominal segment, anterior in position to processes on remaining segments excepting eighth, all spiracles with rounded openings, apparently lined with numerous small vertical carinae, thus margins of openings apparently serrated, eighth segment with spiracles very feebly projecting above surface, openings about 4 mm. apart.

Pupa.—With ***head*** similar to adult excepting for sclerotized transparent enveloping membrane, manifesting two large fleshy but rigid dorsal horn-like processes (fig. 12) projecting forward at each side of median interantennal projection, horn-like processes three times as long as wide, tapering anteriorly and curving laterally, apex acute, outside lateral margin with tooth-like projection bearing a seta just

beyond middle, another similar tooth-like process bearing a seta at external lateral margin near apex; interantennal process bearing a subapical seta at each side; antennal enveloping membrane externally dentate about basal five segments (fig. 13). ***Pronotum*** (fig. 14) similar in outline and punctuation to adult but with setaceous blunt tooth anterior to each posterolateral angle, another setaceous blunt tooth arising at outer anterolateral angle; surface with transverse unevenly margined carina-like ridge appearing just before anterior margin, more elevated laterally, three rather large bluntly rounded setaceous teeth arranged triangularly near outer anterolateral angles at about basal-fourth on each side, pair of tubercles in line with latter teeth but toward middle arranged transversely, rather close together, a pair of small seta arranged transversely, wide apart, slightly posterior to preceding pair of tubercles. *Elytra* and metathoracic wings naturally reduced, apical halves curving ventrally attaining apical margin first abdominal sternite. Abdomen (fig. 16) apparently eight-segmented but ninth fusing with eighth, each of first six segments bearing a pair of spiracles laterally, opening dorsally; lateral processes small, slightly produced, each bearing several hairs, not evident on first two segments, eighth segment with lateral processes, large, elongate, accuminate, acute apically, without setae; tergites with surface microscopically spinulate, but also bearing larger spines in more or less definite patterns, absent on first, second to seventh each with two groups of four spines arranged transversely, one row near cephalic margin, another slightly post-median, cephalic row of spinules larger more widely spread than in post-median row, dorsal-lateral area of each tergite with a single spinule directly in line with lateral process, elevated lateral fold occurring slightly anterior to each spiracle on segments three to seven with distinct spine, all conspicuous larger spines with a short seta arising near base; tail-shovel differing from mature larvae in having prongs more slender, elongate, slightly arcuate, spiracles absent, slightly broader than long, length measured from base of eighth segment to apices of prongs, width between apices of lateral processes, prongs about as long as greatest width between them; sternites four to seven each with transverse row of six to seven spinules just before middle, group of three or four spinules laterally near posterior margin, each spinule with seta arising posteriorly near base. In the pupae studied, the tail shovels of the last larval instar exuvia remained attached to the pupal tail prongs.

This species was confused with *B. chalybeipennis* Zacher by Chujo, for he interpreted specimens from Koror Island of the Palau group as being Zacher's species which was originally described from Ponape Island. Chujo proposed the new genus *Planispa* in 1937 and designated his new species, *P. castaneipennis*, as the genotype. This later proved to be a synonym of *Brontispa mariana* Spaeth, a species described almost simultaneously but having date priority. The other species which Chujo included in *Planispa* was a species he thought to be *B. chalybeipennis*.

Specimens from Koror and Babelthuap Islands agreed entirely with Chujo's redescription of *B. chalybeipennis*, but when compared with the true *B. chalybeipennis*, the Palauan species was quite distinct. *B. yoshinoi* differs from Zacher's *B. chalybeipennis* by having the

interocular structure distinctly broader than long instead of longer than broad, by possessing a narrower interantennal process which is without the thinly produced anterior margins, and by having dark metallic blue elytra as compared to the bluish green elytra of *B. chalybeipennis*. Evidently in Zacher's species sexual dimorphism of the interantennal process does not occur. The pupa of *B. yoshinoi* is easily recognized from that of *chalybeipennis* by the structure of the interantennal process, which is a simple accuminate process in *B. yoshinoi* and bifurcate in *B. chalybeipennis*.

Actually *B. yoshinoi* is probably most closely related to *B. mariana* Spaeth, for they are quite similar in general facies manifesting common characters such as sexual dimorphism of same structures and a broader than long interocular area, but they are easily differentiated as follows: *B. mariana* with brownish elytra, apical area of elytral interstices between the rows of punctures barely raised into costae, mandibles with incisor lobes elongate, overlapping nearly their entire lengths (fig. 6), interantennal projection of *mariana* ♂ broader, longer, exceeding first antennal segment, in ♀ this projection with truncate apex. The mature larva of *B. yoshinoi* has the abdominal lateral processes bearing setae at middle and on the anterior half while *mariana* has at least one seta arising at or near basal fourth of lateral process, and the prongs of the tail-shovel are more strongly dentate dorsally. The pupa of *B. yoshinoi* has the pronotum with three setaceous tubercles on each side of disc near outer anterolateral angles while in *mariana* there are four on each side of disc.

In Maulik's table (1938) *B. yoshinoi* keys out to *B. surigaoana* Uhmann, but the latter species differs in being smaller, in having the interantennal process broader and more truncate apically, in lacking a scutellar row of punctate striations in each elytron, and in coloration.

Since 1938 only a single new species has been described in *Brontispa*, and this species from Namorik atoll in the Marshall Islands was named *B. namorikia* by Maulik (1946). This species superficially resembles and may be confused with *B. yoshinoi*. However, the elytra in *B. namorikia* have a greenish tint, the interantennal projection is very broad and apically truncate, the interocular process is oblong, being nearly as wide as long, and the pupa has the interantennal process bifurcate as in *B. chalybeipennis*.

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ON THE RELATIONSHIPS OF SOME MAMMAL FLEAS TO THEIR HOSTS

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INTRODUCTION

Knowledge of the biology of fleas in their native environments consists chiefly of records of their geographical and host distribution. Information about their life histories and host relationships is limited very largely to that acquired in epidemiological investigations of plague and of other flea-borne diseases. Such work has usually been governed by hygienic and economic necessity and unfortunately has not always been endowed with a broad ecological point of view. Considerable attention has been given to the development of a "satisfactory" flea index, that is, an estimate of the density of flea populations as indicated by samples taken from the hosts, whereas little has yet been done to elucidate the *factors* which determine the density of the population-at-large, of which the individuals collected on hosts may be a small and varying fraction. The paucity of material on the ecology of fleas has prompted us to prepare these somewhat fragmentary notes.

During a survey of small mammals carried out in the years 1936–39 in Bagley Wood near Oxford, England, an opportunity arose to observe the relationships of several flea species to their hosts. This paper is based on observations made at monthly intervals from May 1938 through April 1939, with the exception of July and August. The material is too limited for any very definite conclusions to be made; nevertheless the study presents several points of interest. It was made on a population of live mammals marked for identification and released after each capture; thus the host population was not destroyed and interference with its normal activity was thereby considerably reduced. Changes in the number of hosts and in the number of fleas taken from the hosts were observed simultaneously, so that, if desirable, the two sets of data might be combined to give a more accurate picture of flea infestation and seasonal activity. Records were kept of the flea populations of each individual host at each capture, and of the rapidity with which hosts pick up fleas. Finally, this study was carried out in the same wood in which, ten years earlier, a somewhat similar investigation of parasites of small mammals had been made (Elton *et al.*, 1931); the comparison of results has proved interesting throughout.

The field work was done primarily by one of us (F. C. E.) while a research student at the Bureau of Animal Population, Oxford University, and the identification and study of the flea material by the other

(R. B. F.) while a research student in the Hope Department of Entomology, Oxford University. The determination of flea species was carried out with the aid of Rothschild's synopsis (1915); the nomenclature is that of da Costa Lima & Hathaway (1946), except that *Megabothris* and *Malaraeus* of Jordan (1933) are retained in preference to *Trichopsylla*. We should like to thank the President and Fellows of St. John Baptist College, Oxford, for permission to work in Bagley Wood; Mr. Charles Elton and the Bureau of Animal Population, under whose helpful guidance the field studies were made; Professor P. A. Buxton, Dr. B. M. Hobby and Mr. W. L. Jellison, for valuable suggestions in preparing these notes; and Dr. C. B. Allendoerfer and Dr. C. W. Cotterman for advice on statistical procedures.

PLACE AND METHODS OF STUDY

Bagley Wood lies about four miles from Oxford in a south-westerly direction and is in the county of Berkshire. A more detailed description of the study areas and of the techniques used in trapping and marking the mammals has been published elsewhere (Evans, 1942), but a brief account is desirable here. The area in which these observations were made was one of mixed woodland types, including plantations of larch (*Larix decidua* and *L. kaempferi*), spruce (*Picea sitchensis*) and cedar (*Thuja plicata*), as well as a mixture of these with such broad-leaved species as oak (*Quercus robur* and *Q. petraea*), ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*). The soil was partly gravel and partly clay overlay, and was high enough above the level of the Thames valley and tributary streams not to be flooded at any time of year. All of the study area was well within the wood boundaries, and the nearest human habitations were about half a mile away, so that there was little chance of flea transfer from other areas.

With a grid system of fixed trapping positions which covered two neighboring areas of 6.2 and 4.0 acres, a uniform and strictly comparable sample of the small mammal population was obtained for each month. Animals were trapped alive in Tring traps, baited with cocoanut and provided with nest boxes, and were banded with numbered rings before being released (Chitty, 1937). The weight, sex, approximate age and sexual condition were recorded for each capture. Trapping was continued on each area for four successive nights in each month, the areas being trapped in succession and not simultaneously. The same individuals were often recaptured on two or more trapping nights in each month, and many were caught from month to month. The traps were reset, after removal to another fixed point in the area, immediately after they had been examined and were thus open for 24-hour periods; however, most of the mammals taken were strongly nocturnal in their habits, and it is probable that the great majority of them were not in the traps for more than 12 hours, often for less. Very few rodents died in the traps, but shrews were apparently unable to survive these trap conditions, for they were almost always found dead. Records of fleas from dead specimens were not included in the calculations of flea-indices.

Fleas were removed at each capture. Removal was easily effected by blowing against the fur of the animal, which was held over a white cloth; the fleas collected on the cloth and were transferred to glass tubes.

Each host was examined carefully until it was reasonably certain that all fleas had been removed. A negligible number of fleas escaped by jumping off the cloth. During the warm summer months it was found that fleas sometimes left the host for the nesting material provided in the traps for the mice; this material was carefully searched each time the trap was sprung, and any fleas found in it were attributed to the captured host.

RESULTS

Species composition of the host fauna. The small mammals trapped included three species of rodents and three of insectivores, whose names and zoological affinities are as follows:

ORDER RODENTIA
Family Muridae
Subfamily Murinae
Apodemus sylvaticus sylvaticus (L.), Long-tailed Wood Mouse
Family Cricetidae
Subfamily Microtinae
Clethrionomys glareolus brittanicus (Miller), Bank Vole
Microtus agrestis hirtus (Bellamy), Field Vole

ORDER INSECTIVORA
Family Soricidae
Subfamily Soricinae
Sorex araneus castaneus Jenyns, Common Shrew
Sorex minutus minutus L., Pigmy Shrew
Neomys fodiens bicolor (Shaw), Water Shrew

This assemblage included most if not all of the small mammal species characteristic of the woodland floor habitat of Bagley Wood. However, the field vole was comparatively scarce, and trapping techniques were not designed to insure an adequate representation of shrews. Only the wood mouse and bank vole were caught frequently enough to provide flea data of any quantity.

Species composition of the flea fauna. During the period of this survey a total of 1213 fleas were collected, distributed according to species as follows: *Ctenophthalmus agyrtes nobilis* (Roths.), 1016; *Malaraeus penicilliger* (Grube), 101; *Megabothris turbidus* (Roths.), 38; *Hystrichopsylla talpae* (Curtis), 38; *Palaeopsylla sorecis* (Dale), 9; *Rectofrontia pentacanthus* (Roths.), 4; *Megabothris walkeri* (Roths.), 3; *Doratopsylla dasycnemus* (Roths.), 3; *Nosopsyllus fasciatus* (Bosc d'Antic), 1. All nine species of flea are common in this region and had been previously recorded from Bagley Wood (Elton *et al.*, 1931). This earlier survey obtained three additional species which might have been expected to occur in the present sample but which were not found: *Palaeopsylla minor* (Dale) and *Ctenophthalmus bisoctodentatus* (Kolen.) whose typical host is the mole (*Talpa europaea* L.), and *Leptopsylla silvatica spectabilis* (Roths.), whose typical host is the field vole. A fourth species, *Typhloceras poppei* (Wagn.), has been recorded from the wood mouse in the nearby Tubney Wood (Freeman, 1939).

Of the 650 fleas taken from *Apodemus* and the 556 from *Clethrionomys*, 605 and 411 respectively belonged to one species, *C. agyrtes*. The flea faunas of these two hosts were thus largely composed of a single species. This phenomenon is probably of rather wide occurrence and has been observed in work on many flea populations.

Specificity of host selection. Many species of flea have one particular host species in relation to which their density of population may reach a maximum. Some are more specific than others in their choice of host, though the reason for this may lie as much in the lack of opportunity for host transfer as in any physiological restriction. On the other hand, frequent opportunity for transfer may result in accidental parasitism, such as the occurrence of rodent fleas on predatory birds and mammals, and may falsely suggest a corresponding lack of host specificity. Many small mammal species in a given community appear to utilize the same burrow and runway systems and to compete or co-operate with one another in various ways; this closeness of association may partially account for the lesser degree of host specificity shown by fleas of these animals in comparison with those of larger mammals. During the present study, a large number of burrow entrances were found to be used by both *Apodemus* and *Clethrionomys*, providing abundant opportunity for exchange of fleas. Nevertheless the flea fauna showed considerable range in specificity of host selection.

The different species of fleas were divided into three general groups: (A) those which were constantly present on both the murine *Apodemus* and the microtine *Clethrionomys*, (B) those which are apparently more characteristic of microtine rodents and which occurred on *Clethrionomys*, but either sporadically or not at all on *Apodemus*, and (C) those which were present only sporadically on either *Apodemus* or *Clethrionomys*, and which seem to be primarily parasites of other hosts.

The only species which occurred regularly on both *Apodemus* and *Clethrionomys* and which therefore fell into group A was *C. agyrtes*. This is recognized as by far the most abundant flea on small British rodents, and in this population sample it comprised 83 per cent of the total flea fauna. It was the only species taken in large numbers on *Apodemus*. It is evidently capable of maintaining breeding colonies on *Apodemus*, *Clethrionomys* and *Microtus;* it is also known from many other rodents and insectivores, though it has not been found to form a high percentage of their flea populations (Elton *et al.*, 1931; Freeman, 1939; Matheson, 1934; Thompson, 1938), and for these hosts it may belong to the group C category.

Three species which occurred on *Clethrionomys* and rarely or not at all on *Apodemus* were assigned to group B. Of the 101 specimens of *M. penicilliger* collected, 91 were taken from *Clethrionomys*, on which host it was second only to *C. agyrtes* in abundance. Likewise 29 of the 39 *M. turbidus* and all three specimens of *M. walkeri* came from this host. The latter flea is typically a parasite of *Microtus;* Elton *et al.* (1931) found that 17 per cent of *Microtus* carried *M. walkeri* and only two percent carried *M. turbidus* and *M. penicilliger*, while 17 percent of *Clethrionomys* carried *M. penicilliger*, 12 percent carried *M. turbidus* and three percent carried *M. walkeri*. The two species of one genus, *M. turbidus* and *M. walkeri*, thus appear to occur in different but sympatric habitats, one of which is also occupied by a member of a very closely allied genus, *M. penicilliger*. *Apodemus* had no characteristic species; it is probable that *T. poppei* has this status when it is present in the population (Freeman, 1943).

The remaining five species were taken only occasionally on *Apodemus* and *Clethrionomys* and formed group C. The large *H.*

talpae is by name associated with the mole and is indeed most frequently found on this animal, though it is never numerous on any host; but it can almost certainly maintain itself apart in the nests of small rodents and shrews. It was taken in small numbers from *Apodemus*, *Clethrionomys* and *Microtus*. Large flea larvae, apparently of this species, have been observed in the nests of *Microtus* and adults in copulation have been taken on the fur of *Apodemus* (Elton *et al.*, 1931). O'Mahoney (1939) records this species from Ireland, where the mole is absent. *R. pentacanthus* is found on several species of small rodents and insectivores, but has generally been taken in very small numbers. Freeman (1942) has suggested that species of this genus spend little time on the host and that such records may give little idea of their real numbers. *N. fasciatus* is a cosmopolitan species on commensal rats and the house mouse; a small colony of the brown rat, *Rattus norvegicus* (Erxleben), was present around the cottages at the edge of Bagley Wood, about half a mile from the study area, and the single specimen recorded here was probably a straggler. Both *P. sorecis* and *D. dasycnemus* are typically shrew fleas.

Seasonal distribution of flea species on their hosts. Table I presents the composition of the monthly flea collections from *Apodemus* and *Clethrionomys*. These figures do not indicate population trends, as the number of host records differs for each month, but they do suggest certain seasonal differences in the relationships of the various flea species to these hosts. The group A species, *C. agyrtes*, was at all times the predominating flea on both *Apodemus* and *Clethrionomys*. Group B species were also taken regularly on *Clethrionomys*, but there was a tendency for them to occur on their less frequent host, *Apodemus*, chiefly in the autumn and winter months, when *C. agyrtes* was least abundant. This tendency was even more apparent in group C, whose members were found only in small numbers on either host; of the 48 individuals belonging to the five species of this group, 38 were taken from October through January.

Seasonal differences in flea distribution may be due to a variety of factors. Flea species vary in the amount of time spent on the host; some spend all or most of their adult life there, while others may be chiefly nest or burrow inhabitants. They may also exhibit varying degrees of habitat preference. Jameson (1947) found that some species of fleas from small mammals in Kansas occurred on the host when the latter lived in a given habitat but were absent when the host lived in a habitat of different type. Microclimatic conditions will need to be carefully studied before this problem of distribution can be understood.

Association of flea species on individual hosts. Among the 730 captures of *Apodemus* and the 325 captures of *Clethrionomys* recorded in this study, there were 275 and 178 infestations of fleas, respectively. In 84 of these infestations there were two or more species of flea present; the largest number of species on a single host was four. It is of interest to determine what degree, if any, of interspecific association existed among the various flea species. The test of this association proposed by Cole (1949) requires larger samples than we can provide except in the case of *C. agyrtes* and *M. penicilliger*. On *Apodemus*, these two species occurred jointly 4 times, *C. agyrtes* was present when *M. peni-*

cilliger was absent on 254 occasions, *M. penicilliger* was present when *C. agyrtes* was absent 51 times, and neither species was present on 421 occasions. On *Clethrionomys*, the two species occurred jointly 47 times, *C. agyrtes* was present when *M. penicilliger* was absent 108 times, *M. penicilliger* was present when *C. agyrtes* was absent 15 times, and neither species was present on 155 occasions. The coefficient of interspecific association was found to be $-0.808 \pm .175$ on *Apodemus* and $+0.537 \pm .109$ on *Clethrionomys*. This indicates a strong negative association of *C. agyrtes* and *M. penicilliger* on *Apodemus* and what may

TABLE I

COMPOSITION OF THE MONTHLY FLEA COLLECTIONS FROM APODEMUS AND CLETHRIONOMYS

	Group A	Group B		Group C					
	C. agyrtes	*M. penicilliger*	*M. turbidus*	*M. walkeri*	*H. talpae*	*P. sorecis*	*D. dasycnemus*	*R. pentacanthus*	*N. fascialus*
From *Apodemus:*									
January	59	2	0	0	5	0	0	0	0
February	54	0	0	0	2	0	0	1	0
March	68	0	0	0	1	0	0	0	0
April	84	0	0	0	0	0	0	0	0
May	67	0	0	0	2	0	0	0	0
June	93	1	2	0	0	0	0	0	0
September	88	3	3	0	2	1	0	0	0
October	37	4	2	0	4	3	1	0	0
November	28	0	2	0	0	1	0	1	0
December	27	0	0	0	1	0	0	1	0
From *Clethrionomys:*									
January	51	13	5	0	4	0	0	0	0
February	27	2	1	0	0	0	0	0	0
March	25	5	0	0	0	0	0	0	0
April	27	3	1	0	0	0	0	0	0
May	72	7	3	1	0	0	0	0	0
June	99	10	5	1	0	0	0	0	0
September	23	4	3	0	1	0	0	0	0
October	31	15	7	1	10	1	0	0	0
November	25	9	2	0	2	0	0	0	1
December	31	23	2	0	2	0	0	1	0

perhaps be characterized as a moderate positive association on *Clethrionomys*. The interacting behavior of these two species of flea is apparently quite different on *Apodemus* from that on *Clethrionomys*. Perhaps the fur of *Clethrionomys*, which is somewhat longer and coarser than that of *Apodemus*, may allow *C. agyrtes* and *M. penicilliger* to exist side by side on that host and to avoid such direct competition as may occur on *Apodemus*.

The sex ratio in fleas. It has frequently been observed that more female than male fleas are found in samples collected from rodent

TABLE II

DISTRIBUTION OF CAPTURES OF APODEMUS AND CLETHRIONOMYS SHOWING THE NUMBER OF C. AGYRTES AT CAPTURE

	NUMBER OF *C. agyrtes* AT CAPTURE																					Total Captures	Total Fleas	Mean No. per Capture
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
Apodemus																								
INITIAL CAPTURES	82	24	7	0	3	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	118	63	0.534
SUBSEQUENT CAPTURES	383	114	40	34	16	9	8	1	0	1	2	3	0	0	0	0	0	0	0	0	1	612	542	0.886
Captures after 24 hours	199	69	22	19	10	7	4	1	0	1	1	2	0	0	0	0	0	0	0	0	0	335	317	0.956
Captures after one month	124	32	12	13	1	1	3	0	0	0	1	0	0	0	0	0	0	0	0	0	1	188	152	0.808
TOTAL CAPTURES	465	138	47	34	19	10	8	1	1	1	2	3	0	0	0	0	0	0	0	0	1	730	605	0.829
Clethrionomys																								
INITIAL CAPTURES	57	13	11	9	0	2	2	1	0	1	0	2	0	1	0	0	0	0	0	0	0	99	135	1.364
SUBSEQUENT CAPTURES	120	56	15	6	11	5	5	4	1	1	0	1	0	0	0	0	0	1	0	0	0	226	276	1.221
Captures after 24 hours	25	15	7	3	2	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	57	95	1.667
Captures after one month	71	25	8	3	6	1	3	1	1	0	0	1	0	0	0	0	0	0	0	0	0	120	117	0.975
TOTAL CAPTURES	177	69	26	15	11	7	7	5	1	2	0	3	0	1	0	0	0	1	0	0	0	325	411	1.265

hosts, and in the present study similar findings were noted for the two flea species which were taken in fairly large numbers. The sample of 1016 *C. agyrtes* showed a ratio of 67.9 males per 100 females and that of 101 *M. penicilliger* a ratio of 36.5 males per 100 females. This may of course reflect either a similar inequality in the larger populations of these species, or a difference in the activity and behavior of the sexes, or both. For some species of fleas it is known that the adult life of females is significantly longer than that of males; hence, if the ratio at emergence is not already biased in favor of males, females will tend to predominate. It has also been pointed out that females tend to emerge sooner than males, and if samples are taken early in the development of a colony then females will predominate (Hirst, 1924). Although copulation may take place, in some species at least, between unfed individuals, females do not lay eggs unless they have fed, nor continue to lay unless they continue to feed; the necessity for more frequent feeding may in itself account for the presence of more females than males on the host. Experimental evidence of sex differences in habits has been obtained by placing equal numbers of the two sexes in a nest box occupied by a mouse and finding that significantly more females than males appeared on the host (Buxton, 1938).

Extent of breeding. In the present study, all *C. agyrtes* females were examined for eggs, which can be clearly seen, in the later stages of development at least, in xylol-cleared specimens. Gravid females were found in all months in which collections were made, indicating that there was no cessation of breeding. The percentage of females that were gravid ranged from a low of 20.5 in December to a high of 51.0 in February; there was no clear indication of seasonal variation.

Presence of mites on fleas. Fifty-seven mites, all larval phases of some species of Tyroglyphinae, were found on the fleas. Dr. F. W. Turk, who has very kindly examined some of this material, has identified the specimens as *Tyroglyphus farinae* (L.). These mites were taken from *C. agyrtes, M. penicilliger* and *M. turbidus;* their presence is a case of phoresy, not of feeding on the fleas.

Rate of re-infestation of hosts. It has been shown that rodents from which the flea population has been removed may regain many fleas within a very short time (Meyer, 1938). Quite independently, the present study made a similar observation for *Apodemus* and *Clethrionomys* and gave further indication of the rate at which re-infestation occurs. The only figures suitable for statistical analysis were those for *C. agyrtes*. The following series of typical records gives the number of *C. agyrtes* taken from individual hosts on four successive trapping days:

Apodemus	*Clethrionomys*
0, 1, 0, 4	0, 0, 0, 2
0, 2, 0, 3	1, 1, 1, 2
2, 3, 0, 2	6, 1, 2, 2
0, 3, 0, 3	0, 2, 3, 0
1, 2, 4, 1	3, 0, 1, 0
1, 0, 9, 1	4, 2, 2, 1
0, 0, 0, 5	3, 3, 2, 1
3, 11, 2, —	—, 2, 9, 7
0, 0, 6, 5	1, —, 5, 17
1, 5, 1, 7	0, 5, 1, 11

(— indicates that the host was not captured on that day.)

It is clear that within 24 hours or less hosts may acquire as many or more fleas than they were carrying the previous day. In many cases, fleas were recovered from hosts that had had none the day before, and in one instance 17 fleas were recovered from a bank vole after its previous flea fauna of 5 individuals had been removed the preceding day.

To analyze the rate of re-infestation, the numbers of fleas found on hosts whose recapture occurred approximately 24 hours after previous capture were compared with those found on hosts recaptured after one month had elapsed since previous capture. The distributions of these records showing the number of *C. agyrtes* at capture is given in Table II (Subsequent captures: captures after 24 hours vs. captures after one month). The marked skewness of the data requires their transformation into normal distributions for statistical treatment. It was suggested to us by Dr. C. B. Allendoerfer that this might be accomplished

TABLE III

NUMBERS OF INFESTATIONS AND NUMBERS OF C. AGYRTES TAKEN EACH DAY FROM APODEMUS WHICH WERE CAUGHT ON THREE AND FOUR SUCCESSIVE TRAPPING DAYS

	Successive Days of Capture			
	First	Second	Third	Fourth
FOUR-DAY RECORDS				
Number of captures	33	33	33	33
Number of infestations	13	16	12	23
Per cent infested	39.4	48.5	36.3	69.7
Total number of fleas	19	32	29	47
Fleas per infestation	1.46	2.00	2.42	2.04
THREE-DAY RECORDS				
Number of captures	32	32	32	
Number of infestations	17	20	19	
Per cent infested	53.1	62.5	59.4	
Total number of fleas	59	53	51	
Fleas per infestation	3.47	2.65	2.68	

by substituting a new variable, $Y = \log 1+X$, for the original variable X = number of fleas at capture. The transformed data were then plotted on logarithmic probability paper, with $1+X$ plotted on the logarithmic scale and on the other the cumulative percent of the number of fleas less than X. The results were fairly good approximations to straight lines, indicating that the new distributions were normal. In each such comparison the two lines had nearly the same slope, suggesting equal variances in the two series. Using the transformed variable Y, the significance of the difference between the means for the two series was tested by the usual Student-Fisher t formula. For the data on *Apodemus*, we find $t = 1.38$ (degrees of freedom $= n_1 + n_2 - 2 = 521$), and for the data on *Clethrionomys* $t = 1.92$ (d.f. $= 175$). Neither of these values of t denotes significance at the 5 percent level. Thus there was

no proved significant difference between the numbers of fleas on hosts recaptured 24 hours after previous capture and the numbers present on hosts with an interval of 30 days between captures. The hypothesis that these hosts regained as many fleas within 24 hours as they were found to have after a much longer interval of time need not therefore be abandoned.

The removal of fleas at daily intervals over a four-day trapping period did not apparently result in any decrease in flea abundance. *Clethrionomys* were rarely caught more than two days in succession, but for *Apodemus* there were 32 records of captures on three successive

TABLE IV

MONTHLY DISTRIBUTION OF CTENOPHTHALMUS AGYRTES ON APODEMUS SYLVATICUS

	Number of hosts	Number of infested hosts	Percent of hosts infested	Number of captures	Number of infested captures	Percent of captures infested	Number of fleas	Mean number of fleas per host	Mean number of fleas per capture	Mean number of fleas per infested host	Mean number of fleas per infested capture
January	37	15	40.6	85	21	24.7	59	1.59	0.69	3.94	2.81
February	36	25	69.5	87	35	40.2	54	1.50	0.62	2.16	1.54
March	27	21	77.6	89	31	34.8	68	2.52	0.76	3.24	2.19
April	27	17	63.0	73	33	45.3	84	3.11	1.15	4.95	2.55
May	17	15	88.3	32	25	78.2	67	3.94	2.10	4.46	3.74
June	17	17	100.0	42	27	64.4	93	5.47	2.22	5.47	3.44
September	28	20	71.5	43	33	76.6	88	3.14	2.04	4.39	2.66
October	35	20	57.3	79	25	31.6	37	1.12	0.47	1.85	1.48
November	48	15	31.2	95	20	21.0	28	0.58	0.29	1.87	1.40
December	45	14	31.2	104	15	14.7	27	0.60	0.26	1.93	1.80
ENTIRE PERIOD OF STUDY											
♂♂ hosts	56	42	75.0	319	140	43.8	328	5.85	1.03	7.80	2.34
♀♀ hosts	62	42	62.7	411	125	30.4	277	4.46	0.67	6.60	2.22
Totals	118	84	71.2	730	265	36.3	605	5.14	0.83	7.21	2.28

days and 33 additional records of captures on four successive days. The number of infestations and the numbers of *C. agyrtes* taken each day for these records are shown in Table III. No marked decline is evident. If captures had been repeated over longer periods or at shorter intervals the number of infestations and the number of fleas per capture might well have decreased.

These facts suggest that individual fleas did not spend any prolonged period on their hosts. Freeman (1945) has shown that *N. fasciatus* left their hosts in considerable numbers every day, at a feeding place which their hosts were visiting regularly. It is likely that nests and burrows of the hosts are the principal foci of these flea populations.

No data are available from nests of *Apodemus* or *Clethrionomys*, but an analysis of the fauna of 38 *Microtus* nests gave counts of as many as 92 fleas per nest (Davis, 1934). In our study, the greatest number of *C. agyrtes* taken in a single infestation was 20 (Table II). As a rule, however, the number was very much lower and about 50 percent of the infestations were made up of single specimens. Clearly the maximum or potential "flea-holding" capacity of the host was rarely if ever attained. It was also evident that *C. agyrtes* was not distributed at random among the captures of either *Apodemus* or *Clethrionomys*. This was tested by fitting a Poisson series to the data in Table II for the total captures of

TABLE V

MONTHLY DISTRIBUTION OF CTENOPHTHALMUS AGYRTES ON CLETHRIONOMYS GLAREOLUS

	Number of hosts	Number of infested hosts	Percent of hosts infested	Number of captures	Number of infested captures	Percent of captures infested	Number of fleas	Mean number of fleas per host	Mean number of fleas per capture	Mean number of fleas per infested host	Mean number of fleas per infested capture
January	21	17	81.0	47	25	53.3	51	2.43	1.09	3.00	2.04
February	15	9	60.0	23	9	39.2	27	1.80	1.17	3.00	3.00
March	9	5	55.6	15	8	53.4	25	2.78	1.66	5.00	3.12
April	8	7	87.5	11	8	72.8	27	3.38	2.46	3.85	3.34
May	14	8	57.2	22	13	59.0	72	5.15	3.28	9.00	3.27
June	17	15	88.3	28	24	85.5	99	5.73	3.42	6.60	4.12
September	29	10	34.6	37	10	27.0	23	0.79	0.62	2.30	2.30
October	46	17	37.0	59	17	28.8	31	0.67	0.53	1.82	1.82
November	34	13	38.2	40	13	32.5	25	0.74	0.62	1.92	1.92
December	31	15	48.5	43	21	48.9	31	1.00	0.72	2.07	1.48
ENTIRE PERIOD OF STUDY											
♂♂ hosts	58	44	75.9	199	97	48.8	302	5.21	1.52	6.88	3.12
♀♀ hosts	41	29	70.8	126	51	40.5	109	2.66	0.87	3.76	2.14
Totals	99	73	73.8	325	148	45.6	411	4.15	1.26	5.63	2.78

each host (Snedecor, 1946, p. 441); the deviation from the Poisson series tested by chi-square indicated that, when captured, some individual hosts were more heavily infested than others. Variations in the number of fleas available for infestation, due to differences in the flea populations of the various burrows at various times of year, seem to afford a more plausible reason for this non-random distribution than differences in susceptibility or in activity of the hosts.

Flea indices. *C. agyrtes* was the only species of flea which we took in sufficient numbers for the calculation of indices of relative abundance. Tables IV and V present the data for this flea on *Apodemus* and *Clethrionomys* respectively.

In the ordinary type of flea survey employed by epidemiologists and others, indices are usually based on collections from hosts which are caught once only and which are thereby removed forever from the host population, thus possibly affecting future host-parasite relationships. The technique used in the present study avoided this possibility and provided a series of collections from hosts which were caught and recaught varying numbers of times. In the latter technique, initial captures would correspond to those obtained by ordinary survey methods. By grouping our data according to initial and subsequent captures, it is possible to compare the two methods of collection. The distribution of records showing the number of *C. agyrtes* at capture is presented in Table II (Initial captures vs. Subsequent captures). For each host, the data were transformed as described above and were then subjected to the Student-Fisher t test. For the data on *Apodemus*, we find $t = 2.11$ (d.f. $= 728$), and for the data on *Clethrionomys* $t = 0.154$ (d.f. $= 323$). The t value for *Clethrionomys* indicates no proved statistical difference between the two series; that for *Apodemus* indicates a significant difference at the 5 percent level but not at the 1 percent level. The data are not very extensive but, if they are accepted as adequate samples, the comparisons do not reveal any striking differences between the results of the two methods of collection. It is evident, however, that they do not always give similar results.

For each species of host, the monthly indices point clearly to a rise, not only in the number of fleas per host, but also in the numbers of hosts infested, during the spring, with a maximum reached in the summer and a subsequent decline to a winter low. Such seasonal variation has been observed repeatedly, yet it is by no means clear how much this is due to a real decline in the numbers of fleas present in the breeding environment (the nests and burrows of the hosts), how much to a decrease in flea activity, or how much to changes in the numbers or activity of the host population. The presence of certain flea species on occasional hosts primarily in the autumn and winter months, which was observed in this study, suggests seasonal differences in habits, perhaps of host as well as of parasite. Changing conditions of temperature and humidity probably also bring about differences in activity, but again we lack definite information. The absence of specific knowledge about such points as these re-emphasizes the need for further intensive study of flea ecology.

SUMMARY

1. During a survey of small mammals near Oxford, England, fleas were collected regularly each month from May, 1938, through April, 1939, except for July and August. The hosts were taken alive and released for subsequent recapture; all fleas were removed at each capture.

2. Nine species of fleas were recorded, taken almost wholly from the wood mouse, *Apodemus sylvaticus*, and the bank vole, *Clethrionomys glareolus*. A single species, *Ctenophthalmus agyrtes*, accounted for 1016 of the 1213 individual fleas collected.

3. The various flea species were arranged in three groups: (A) those which were constantly present on both *Apodemus* and *Clethri-*

onomys, (B) those which occurred on *Clethrionomys*, but rarely or not at all on *Apodemus*, and (C) those which were present only sporadically on either *Apodemus* or *Clethrionomys* and which were apparently primarily parasites of other host species.

4. When fleas of group C did occur on *Apodemus* and *Clethrionomys*, it was principally in the autumn and winter months, when *C. agyrtes* was least abundant.

5. As many as four species of flea were obtained on an individual host. *C. agyrtes* and *M. penicilliger* showed a strong negative association on *Apodemus* and a moderate positive association on *Clethrionomys*. It is suggested that fur differences between the two host species might account for this difference in association.

6. The sample of 1016 *C. agyrtes* showed a sex ratio of 67.9 males per 100 females, and that of 101 *Malaraeus penicilliger* a ratio of 36.5 males per 100 females.

7. Gravid females of *C. agyrtes* were found in all months in which collections were made; the percentage of females that were gravid ranged from a low of 20.5 in December to a high of 51.0 in February.

8. Mites of the species *Tyroglyphus farinae* were found as "riders" on *C. agyrtes*, *M. penicilliger* and *Megabothris turbidus*.

9. Individual hosts from which the flea population had been removed were found frequently to acquire in 24 hours or less as many or more fleas than they were carrying the previous day. It is likely that the principal focus of the flea populations lay in the nests and burrows of the hosts rather than on the hosts themselves.

10. The method of collecting fleas described herein was shown to provide indices not strikingly different from those obtained by ordinary survey techniques. Indices of relative abundance derived for *C. agyrtes* showed a definite rise in the spring and a decline in the autumn and winter months.

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KURS OBSHCHEI ENTOMOLOGII, by B. N. SHVANVICH. Sovietskaia Nauka. 900 pages, 590 text figures. Price, 30 rubles.

This work marks the appearance of a text in the Russian language which may be considered comparable to the well-known works of Comstock, Imms, and Henneguy, though differing from them somewhat in scope. It is hardly as ambitious a publication as Berlese's *Gli insetti* or Schröder's *Handbuch der Entomologie*.

Shvanvich's text, in general, makes a favorable impression. The stress is on insect morphology, in which field the author draws heavily from Snodgrass; he also places considerable emphasis on physiology, and the physiological and morphological parts seem to be well integrated, both phases in many instances being considered together. Indeed, the work is so predominantly morphological and physiological that other phases of entomology receive comparatively little attention. An interesting feature is the discussion of coloration and the evolution of color patterns, based to a considerable extent upon the author's original investigations, and to which 52 pages of the text are devoted. This discussion leads logically to a consideration of protective resemblance and mimicry. Embryology, insect life histories, and metamorphosis are given due consideration, but to find any material dealing with ecology, human relationships of insects, or most phases of insect biology, one must, except for the brief foreword, look in scattered places throughout the text. The treatment of taxonomy is brief but concentrated. The one-sided emphasis hardly fits the title, which may be translated, "A course in general entomology".

It is difficult to determine how much original work has gone into the text. An extensive list of citations (26 pages) is appended, and American and Western European authors are given their just representation. Here again, taxonomic works, including some important ones from the Russian literature, are neglected. However, documentation is sparingly used. Credit is given for many of the figures and some are marked "Orig." (original), but many, among which one may recognize familiar illustrations, are used without credit. In the foreword the author does state, however, that such illustrations are taken from either Snodgrass or Weber.

The printing and reproduction of illustrations is good; the paper and binding only fair, though better than that used in many recent Russian publications. We secured a copy from an American dealer for about $5.00.—M. T. J.

OLFACTOMETER EXPERIMENTS WITH MALE BRACONIDS

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The parasitic wasp, *Microbracon hebetor* (Say) is well known in biological control as a very useful enemy of lepidopterous pests in stored grains. The wasp is also well known for its usefulness in genetic investigation as evidenced by the fact that Dr. P. W. Whiting and associates have published genetic studies on the organism (as *Habrobracon juglandis* (Ashmead)) for over 30 years (Martin, 1947). Important factors in the maintainance of braconid stocks which should be of interest to both groups of biologists concern the behavior of males in the presence of possible mates and in the presence of potential food.

Grosch (1947, 1948) found evidence of an olfactory basis in mating behavior. When a search of the literature failed to disclose olfactometer experiments with *Microbracon* males, experiments using olfactometer apparatus were instituted to investigate mating attraction. Also, with the same equipment, investigation was extended to a survey of the attractive ability of various substances which might give clues to male food preferences. Thus the present report gives information bearing on the basic question: What objects are sufficiently attractive to cause a male wasp to seek them? In addition, specific methods of investigation are developed and observations are presented on correlative behavior in starved and unstarved males.

MATERIALS AND METHODS

The normal type of males which are produced parthenogenetically as haploids were used in all experiments. To obtain these impaternate males, virgin females of an eugenic wild type strain were placed with healthy host caterpillars. Ten days later during the expected time of emergence from cocoons, culture vials were examined morning and evening. Males which had emerged since the preceding examination were removed and placed in separate containers labelled with the time and date of collection. Thus the age of males within a group was known to the nearest 12 hours.

Cultures were maintained and all experiments were performed in a windowless cubicle (6x5x7 ft.) the temperature of which was maintained at 86° ± 1° F. A maximum-minimum thermometer was used to record temperature. To guard against dried atmosphere a dishpan was kept filled with water from a dripping tap. Attempts to record humidity were not made but no change from these standard culture conditions occurred during the series of experiments.

Representative types of Y-tube olfactometers (see Peterson '47) were made available by Dr. W. M. Kulash of the North Carolina State College Zoology and Entomology Department. Use of the equipment

was reluctantly discontinued after repeated attempts to set up satisfactory systems failed. Various difficulties prompted this decision. In the first place (1) male wasps were found to have an extremely strong "escape" instinct. That is, they can and will force themselves out of an experimental system through very small crevices. This makes a suction outlet undesirable, for, even when plugged with cotton, any passage which allows entrance of a draft of air enables males to leave the field of investigation. Secondly (2), shadows or dimness act as barriers to free movement. Males are definitely slowed and sometimes even stopped by areas of shade or shadow. This fact argued against the use of a light source to furnish the stimulus to bring test wasps to the place of choice. Furthermore, (3) starved males when first freed in a system are too active to serve for orientation records. They give the impression of indiscriminate trial and error exploration for an escape route. (4) Males are found to walk rather than fly in containers. Thus a two dimensional route is followed on the walls of the experimental system. This is reflected in a definite correlation between the side of the lead tube traveled and the arm into which the wasp turned at what was intended to be a point-of-choice. For this reason direction of first turn is of little value except perhaps with the small number of animals which pause at the point of choice.

With these considerations in mind a closed system was developed which could be used in the dark. This consisted of a simple T-shaped tube which was clamped with the center arm in the vertical position. To the lower end of the vertical arm was attached a vial containing test males. The male wasps reacted by negative geotropism to move up the vertical tube (60 mm. in length) to the point of choice from which horizontal arms (each 60 mm. in length) lead to trap vials. Odorous materials to be tested were placed in one or both trap vials. These trap vials (15 by 70 mm.) were fitted with corks carefully bored to receive an end of the horizontal tube. With the tubing attached, the vial was corked with a square of gauze so fitted that the edges are tightly held between cork and vial but a slack barrier of gauze extends into the vial. This functions as a one way valve. Wasps can force themselves into the trap because the gauze is held firmly against pushing movements in that direction. Escape is not easy because the gauze is thrown into loose folds if an animal attempts to push through in the opposite or "out" direction.

After a desired period of time the lights can be turned on briefly to enable the recording of the number of wasps in each trap. If sufficient numbers are employed, the distribution of males should indicate an attraction or repulsion by the presence or absence of a significant number of animals with the test material, or the lack of directive stimulus by random distribution.

The T apparatus was first used with females as the lure in one trap and nothing in the other. This and all the following experiments were run for four hours. At the beginning of each experiment the light remained on for five minutes in order that observations might be made which could be compared with those on behavior in Y tube apparatus. The second series of tests compared the reactions of males when honey, a routine laboratory food, was present in one trap and females were

appraised as the lure in the other. By employing males of known age, the effect of starvation on the behavior of males was considered in this set of experiments. Groups used were unfed males 1, 2, 3, 4, 5, and 6 days old.

After the honey-*vs.*-female experiments a qualitative survey was made of the attractent-repellent ability of a representative series of odorous substances by placing the material in one trap vial and having the other trap empty. Sweet substances, fermentation products, fruits, flowers, and a miscellaneous series of odorous materials were thus screened. The assortment of fruits and flowers available was limited by the time of year (spring) at which the experiments were performed. A general plan was to include at least one member of each class of the odors catalogued in Moncrief (1946), Chapter 18, entitled "Classification of Odours."

Dilute suspensions, needed when testing strongly aromatic compounds, were made by violently mixing 0.1 cc. of the material with 10 cc. of distilled water. A drop from the end of a dissecting needle was immediately placed in the desired trap vial before the suspension separated. If more permanent dilutions are desired a stabilizing agent (such as gum tragacanth) should be employed to maintain suspension. Because the present survey was intended to be only qualitative in nature the dilutions were merely the means of delivering a very small amount (or, in the case of alcohols, a graded series of amounts) of a given substance. No importance is meant to be given to the absolute quantitative value of the dilution.

After a test, apparatus had to be washed, dried, and aired thoroughly before re-use. Furthermore, tests run with used squares indicate that gauze squares should be discarded after each test in order to eliminate confused results due to residual odor.

RESULTS AND CONCLUSIONS

Females as the lure.—Summarized results for five tests using day-old males (10 per test) show 14 males in the trap with females, 16 males in the lureless trap, and 20 males scattered at random in the T tube and the original vial. This random distribution occurred in spite of the fact that before the light was turned off a majority of males (73%) flipped their wings in the characteristic manner known as the mating reaction (Whiting 1932) at the mouth of, or within the tube leading toward the trap containing females. These mating reactions were followed by excited ambulatorial movement which however was not consistently directional. (Incidentally, comparable indications of recognition of female presence had been observed in classic Y tube apparatus.)

Starved males used in a similar series of experiments gave similar results. That is, no significant number of males were attracted into the female vial and the observed distribution appeared to be at random.

A series of experiments in which males were offered the choice between a trap vial with 10 females and a trap vial with a small drop of honey gave the following results. Day-old males (10 in each of five tests) after 8 hours of darkness were found distributed as follows: 23 males with females, 22 males with honey, 5 males not in traps. This

random distribution in the traps seems comparable with the female-*vs.*-zero trap vial results above. When starved males were used, striking differences were found in resultant distributions. All the males were then found in traps, and, as starvation increased, more and more males were found with the honey. Summarized results for five experiments at each age:

For 2-day-old males: 30 in honey trap, 20 in female trap.

3-day	34	16
4-day	43	6
5-day	50	0

After 6 days or more of starvation males showed signs of weakening and some died. Although their reactions were limited by their failing strength they were extremely excitable. In one case, three males which had been starved for 6 days were able to make such rapid and apparently effective directive movements that they entered the honey trap and began feeding within the first second after introduction into the system, even before the light was turned out.

In order to see what might happen if starved (5 days) males passed close to females during their movements, gauze barriers were omitted from trap mouths and the light kept on while observations were made in an additional experiment. Six of the males came up the vertical arm of the T in the first five minutes; after slight hesitation four of them turned down the tube leading toward the honey. The other two had not hesitated (had not made a choice?) and their movements followed the surface on which they were progressing. This led them into the female arm. After a few millimeters one male stopped, waved his antennae as if testing the air, then turned to go in the direction of the honey. Meanwhile three females had emerged from their vial and had approached within 6 mm. of him. Suddenly he stopped, flipped his wings in the mating reaction, rushed to a female, mounted and copulated. The second male by this time also recognized the presence of females by giving the mating reaction and attempted to mount the same female. The two unmated females moved on toward the honey. When they came within 6 to 10 mm. of the other five males these also turned, gave mating reactions and attempted mating. It seems worthy of note that all these mating activities occurred before the starved males had fed. After these observations had been made the light was turned off and the system remained in the dark for four hours. At the end of that period it was found that the males had remained in the honey vial while the females had moved *down* to the lowest location in the experimental system. This was the original male vial reached by way of the vertical tube.

Attraction to various materials.—Tables I and II present the summarized results of a qualitative survey on males starved 5 days. For cases in which the total number of wasps recorded in both test traps is shy of the total males tested, the number lacking had not entered the traps. Usually these males were found in the original male vial.

In many cases the results show certain consistent deviations from a random assortment. The chi-square test can be applied to questionable cases where inspection does not suffice. It is evident that honey proved

to be the most powerful attractent while beeswax did not have similar attributes. Karo sirup and molasses were almost equal to honey in attractent potency but fresh sugar sirups lacked similar ability. Along with these observations it was interesting to find that a faint alcoholic odor attracted *Microbracon* males but acetic acid, acidic fluids, and sour fruits did not. The wines used had odors which for human sense of

TABLE I

DISTRIBUTION OF MALES IN OLFACTOMETER TRAP VIALS

Ten tests—total 50 males in each experiment

MATERIALS TESTED	NO. WITH MATERIAL	NO. IN ZERO TRAP
Sweets		
Honey	42	8
Karo	40	10
Molasses	38	9
Brown sugar sirup	21	29
Sucrose sirup	26	24
Milk chocolate	17	33
Fermentation Products		
Alcohol (Ethyl) 95%	10	40
Alcohol (Ethyl) 70%	12	38
Alcohol (Ethyl) 50%	28	20
Alcohol (Ethyl) 30%	32	18
Alcohol (Ethyl) 5%	38	12
Acid (Acetic) 0.1%	0	33
Wine (Scuppernong) 14% Alcohol	14	36
Wine (Sherry) 20% Alcohol	0	30
Vinegar (Cider) conc.	0	50
Vinegar (Cider) 0.1%	28	22
Fruits		
Strawberry (fresh)	16	34
Strawberry (sour)	0	50
Banana (fresh)	26	25
Banana (sour)	0	43
Apricot (dried)	31	19
Fig (dried)	30	8
Date (dried)	30	18
Flowers		
Clover (white)	8	42
Rose (red)	0	50
Privet	0	50
Wild Carrot	6	42
Honeysuckle	0	50

smell completely masked any odor contributed by a low alcohol content. A similar effect for the insects would possibly explain why the substances did not prove attractive. Flowers available at the time of the year when the experiments were made, although frequented by bees and other insects had no attractent power for *Microbracon* males. None of the miscellaneous odors, with the possible exception of oil of bergamot

(dilute), attracted males. In fact, cases in which all males are in the zero trap may be interpreted as indication of repellent action.

Used gauze squares when tested for residual odor showed a positive attraction with honey, karo and molasses and a repellent action with such substances as proved repellent in Tables I and II except in the case of terpineol (lilacine). The residual odor of this substance proved attractive in the extremely faint odor given off by the used gauze.

Conclusions on male food habits.—Present evidence on behavior of *Microbracon* males indicates that on the basis of olfactory attraction the source of food is limited to a single type-group comprising rich sirups and sirupy fruits. Attractiveness of a faint odor of ethyl alcohol

TABLE II

DISTRIBUTION OF MALES IN OLFACTOMETER TRAP VIALS

Two tests—total 20 males in each experiment

MATERIAL TESTED	NO. WITH MATERIAL	NO. IN ZERO TRAP
Miscellaneous		
Amyl-acetate (dil.)	0	19
Balsam (flakes)	10	10
Beeswax (flakes)	11	9
Bergamot Oil (dil.)	12	6
Cedar Oil (dil.)	0	20
Cheese (mild Amer.)	0	18
Clove Oil (dil.)	0	15
Coffee (ground)	10	10
Lemon Oil (dil.)	5	15
Meat (decomposing)	0	19
Menthol (crystals)	0	20
Peppermint Oil (dil.)	0	20
n-Propyl Formate (dil.)	0	20
Quinoline (dil.)	7	13
Sassafras Oil (dil.)	8	12
Spearmint (leaf)	0	20
Tar (flakes)	0	20
Terpinol (dil.)	0	20
Vanilla (extract 37% alcohol)	6	14
Yeast (cake fragments)	7	13

is consistent with this grouping because insects which feed on natural sirups are accustomed to encounter primary products of fermentation (Dethier 1948). This may hint at the common basis of attraction shared by these enumerated potential food substances. It seems possible that their attractive principles are associated with sugar decomposition even prior to alcohol formation. At any rate this is true in the case of the codling moth, another sirup feeder for which the appearance of acid renders a fermenting mixture repellent (Eyer 1931, 1934). The sirup feeder is quite different in habit from the frequenter of fermenting substances. The fruit fly, *Drosophila*, is an example of the latter. Acids and acetates enhance bait attractiveness and *Drosophila* find cider vinegar and sherry wine very attractive (Barrows 1907) while *Microbracon*, as shown herein, do not.

Sugar content, presumably valuable from the standpoint of utilizable nutriment, is valueless in attracting *Microbracon* males. Olfactory response is lacking with fresh sugar solutions. Successful laboratory feeding of braconids with sugar water seems to depend on a recognition of food by taste and therefore must involve contact stimuli. Sirups (such as honey) which attract from a distance should be more efficient in feeding cultures. If sugar water is the only food available, routine use of small culture vials is recommended in order to favor chance contact with the food.

It is unlikely that males in their normal habitat find food in granaries, flour mills, and similar establishments. Furthermore, they are unable to continue sustained flight to other buildings more likely to contain stored sirup. However, since 5 days of starvation were found to merely increase activity it seems that there is sufficient time during which any available females will be found even by undirected male explorations. In commercial buildings where miscellaneous foodstuffs are stored, success in finding food is more likely. Hamid (1940) has presented similar conclusions.

Non-specific mating reactions.—To distinguish unexplained wing-flipping in the absence of the female or female products, Grosch (1948) suggested the term "non-specific mating reaction." The simplest hypothesis offered in explanation was that non-specific mating reactions were nonpurposive expressions of excitable male reactivity. Observations during the present series of experiments seem consistent with such an explanation. By an avoiding reaction aromatic oils are shown to be repellent, yet when a vial of males is plugged to a test system which contains strong odorous oils a common male reaction is wing flipping similar to that of a true mating reaction. This was especially noticeable with clove oil and was more pronounced and unanimous when concentrated oil was used. Concentrated oil is not suitable for test conditions it was discovered early in preliminary experiments. Males exposed in closed system show high mortality and therefore irritant action of its fumes is suspected. Dethier (1948) points out the interesting parallel that the odor of clove oil quickly kills ants.

DISCUSSION

The escape instinct of small wasps necessitates the use of tightly closed systems when working with the animals. Furthermore since access to stored foods is likely to be by way of minute passageways, the use of closed systems involving tubing of a relatively small diameter seems applicable to investigations of food habits. However, graded intensity of stimulus cannot be well maintained and therefore closed systems seem unsuited for investigations of mating attraction. Mating reactions which apparently are specific, indicate some perception of female presence in the system, yet subsequent random distribution of males implies a failure in the attractive mechanism. It seems that unless the male is relatively near the female, perception of her presence increases trial and error exploration of the system rather than leads him directly to her. These observations in the present apparatus are consistent with observations on smaller closed systems already reported (Grosch 1948).

In order to make an indirect check on the efficiency of mating attraction ten simple tests were made using a 32 oz. jar (height 240 mm., diameter 160 mm.), the largest closed container available. Compared with the size of the animal (length 2 mm.) this is a comparatively large volume which should make chance of meeting improbable. For each test a single virgin was placed in the jar with a single male and the closed jar immediately stored in the dark for 4 hours. At the end of that time the animals were removed and the female was given host caterpillars. In all cases the hosts were parasitized successfully, and when adults appeared the occurrence of biparental offspring indicated satisfactory mating in each test. Does this indicate that a graded intensity of olfactory stimulus can obtain and function adequately in a closed container of relatively large volume?

Unfortunately the chemistry of insect sex scents is virtually unknown (Dethier 1948). In fact no sure human identification of insect sex scent has been reported even in cases as well grounded as certain of the Lepidoptera. Undoubtedly braconid female scent is delicate and tenuous, yet it was interesting to see males which were seemingly intent on reaching honey turn and make directly for females when the potential mates had come within what appears to be the most effective radius of the stimulus. Instead of the conclusions that hunger drive is stronger than mating attraction, an inference which would be made on the basis of trap vial results, these mating attempts by unfed males indicates a possibility that a heavy scent such as honey has a masking effect on sex scent. Strength of the effective stimulus of female scent increases with decreased distance so that mating is observed to be the strongest drive with a suitable strength of stimulus. This involves a relatively close approach of the animals before the attractive mechanism functions effectively. However, in the normal habitat when males and females congregate at a sirup supply a combination of events similar to these observed with females freed in the test system could be an efficient means of perpetuating the wasps.

SUMMARY

1. After unsatisfactory trials with air-draft, light-source olfactometers, a closed system was adopted which should be used in the dark. Negative geotropism of the males is used to bring them to the point of choice. This method is probably not applicable to investigations with females because they are suspected to react by positive geotropism. Difficulties with classic olfactometers are listed.

2. Although males gave some recognition of female presence by wing flipping and an increased rapidity of movement, directive attraction did not function effectively. This is consistent with other findings with closed systems of limited dimensions. In contrast, when a relatively large closed container was used, males unfailingly found virgin females for successful mating. This was evidenced by the biparental offspring produced.

3. Starving males were definitely attracted to honey, karo, molasses, and sirupy fruits. This reaction was more pronounced with greater length of starvation time. Fresh sirups made with simple sugars had no attractive power. A faint odor of alcohol (ethyl) proved attractive

while acetic acid, acidic fluids, and sour fruits were repellent. Flower odors and a representative series of aromatic materials were unattractive. It is concluded that food interest may be limited to a single type-group and suggested that the primary break-down products of sugar decomposition may furnish the attractive principles.

4. Starved males mated with females before feeding if the distance between animals chanced to narrow to less than 10 mm. Contact stimuli were not involved in preliminary attraction.

5. In cases of irritation by aromatic fumes, non-specific mating reactions were observed. This contributes to the hypothesis that these non-specific reactions are nonpurposive expressions of an excited state.

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THE MAYFLIES OF FLORIDA, by LEWIS BERNER. Univ. Florida Studies, Biol. Sci. Series, vol. 4, no. 4, xii+267 pages, frontispiece, 19 maps, 88 textfigures, 24 plates. 1950. Price, $5.50.

The title of this work may imply that it is a taxonomic study, but actually it is much more than that. A glance at the work is sufficient to indicate that the author gave considerable time and effort to the study of distribution, ecology, habits, and life histories of these insects. This work, consequently, will be of considerable interest to the ecologist, the limnologist, and the aquatic game biologist.

A collection of fifty thousand specimens, made from all parts of the state, and over a period of seven years, supplied the taxonomic material. The keys, illustrations, and the brief section devoted to taxonomy under each species, should serve to identify the species, several of which, however, are indicated only by key letters or numbers. It is noteworthy that the key to the nymphs includes all species discussed in the text except one of rare occurrence. It is gratifying to learn that a fauna could be so thoroughly known in its immature stages.

The work presents a very good appearance. It is well printed on a good-grade, smooth paper, which is not, however, so glossy as to produce eye-strain. The half-tone plates are reproduced well, and the maps present at a glance the distribution within the state. A list of references (pp. 255–259) and an index are included.

—M. T. J.

THE GENUS SCOPEUMA IN THE WESTERN UNITED STATES AND SOUTHWESTERN CANADA[1]

(Diptera, Scopeumatidae)

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Malloch (1935) reviewed the New World species of *Scopeuma* (as *Scatophaga*) and included in his review, for purpose of comparison, a discussion of some related Old World forms. His work has, for the most part, been quite serviceable for the identification of American species. The purposes of the present paper are mainly to clarify some matters in regard to synonymy, to present the description of a new species, to discuss distribution, variation, and relationships, and to present a key which, though borrowing much from that of Malloch, should prove somewhat more serviceable for the area covered and at the same time help to indicate relationships.

The name *Scopeuma* was proposed by Meigen (1800), with inclusion of seven species, none of which was indicated by name. Later, Meigen (1803) discarded that name and used in its place *Scathophaga*, with but one included species, *Musca merdaria* L. Coquillett (1910) designated *M. merdaria* as the type of *Scopeuma*, thus making the two names absolute synonyms (*Scatophaga* is merely an emendation of *Scathophaga*). Curran (1934), therefore is not justified in using the name *Scopeuma* for those species with the pteropleura hairy and *Scatophaga* for those with the pteropleura bare.

Malloch characterizes the genus as follows: "Sternopleura with one strong posterior bristle; stigmatal and propleural bristles not or poorly differentiated from the adjacent hairs; palpi without a long apical bristle; fore femur without anteroventral bristles; fore tibia without short stout ventral setulae, and without a well-developed preapical anteroventral bristle; hypopygium of male not bearded; first wing-vein bare [;] sixth vein complete."

The present paper deals with the species of *Scopeuma* recorded from the eleven Rocky Mountain and Pacific Coast States, namely, Washington, Oregon, California, Idaho, Montana, Wyoming, Nevada, Utah, Arizona, Colorado, and New Mexico, and from the two Southwestern Canadian Provinces of Alberta and British Columbia. Of the fifteen species treated here, three are extralimital, but are included because of their possible occurrence in the area in question. *S. pallidum* occurs in the Eastern United States, *S. vittatum* in Mexico, and *S. crinitum* on the Alaska coast. It is interesting to note that, while on the one hand three species, *S. furcatum*, *S. suillum*, and *S. stercorarium* are of Holarctic or even wider distribution, one other, *S. orcasae*, is known from only a small island in Puget Sound.

[1]I am indebted to Mr. Curtis W. Sabrosky for critically reading this manuscript.

This study is based on material from several collections, but particularly from the United States National Museum, the California Academy of Sciences, the American Museum of Natural History, the Chicago Museum of Natural History, the University of Kansas, the University of Colorado, Colorado A. & M. College, the Utah State Agricultural College, the State College of Washington, the University of California, and the University of Idaho.

KEY TO THE WESTERN SPECIES OF SCOPEUMA

1. Pteropleuron with some fine erect hairs on at least a part of its surface..... 2
   Pteropleura entirely devoid of erect hairs..... 7
2. Arista with some long hairs on the basal half, the longest of these about half the width of the third antennal segment; fifth abdominal sternum of male with posterior angles strongly produced and in addition with a pair of approximate apical median processes; antennae entirely deep black; femora for the most part black; cross-vein r-m infuscated, **stercorarium**
   Arista almost or quite bare, or with hairs which are no longer than its basal diameter; fifth abdominal sternum of male with lateral angles strongly produced and divergent, but without apical median processes... 3
3. Presutural acrosticals weak, arranged in four or more irregular series; abdominal pile short, the erect discal pile usually no longer than third antennal segment and not, or but slightly crinkly; lateral lobes of fifth sternum strongly convex inwardly, consequently diverging U-shaped..... 4
   Presutural acrosticals strong and long, arranged in two series; supraspiracular convexity of pleurotergite[2] downy or pubescent, without outstanding fine erect hairs; abdominal hair, especially in male, long and crinkly, at least some hairs longer than third antennal segment and curled at apex; lateral lobes of fifth sternum feebly convex inwardly, consequently diverging V-shaped..... 6
4. Supraspiracular convexity of pleurotergite with erect fine hairs, the longest of which are about as long as the fringe of the lower squama; palpus black, the basal fourth or more usually yellowish..... **molle**
   Supraspicular convexity of pleurotergite downy or pubescent, without outstanding fine erect hairs..... 5
5. Palpus blackened on apical half or more, slightly flattened, and rather slender, being distinctly narrower than front basitarsus; abdominal pollen unicolorous; larger species, 4.5 to 6 mm.; adults flying in summer..... **griseum**
   Palpus yellow, at most darkened at extreme apex, more flattened, and broader, the maximum width almost equal to that of front basitarsus; abdomen grayish, with the bases and apices of segments two to five inclusively distinctly brownish; smaller species, 4–5 mm.; adults occurring in late fall and early spring..... **hiemale**
6. Hairs of thorax and abdomen long, fine; acrosticals usually long, slender, hair-like; scutellum normally with more than two pairs of marginals and submarginals; clypeus[3] black, much darker than parafacials, obscured by grayish pollen; legs black, with grayish pollen; palpus slender, but feebly clavate, usually darkened at apex, usually about 1.4 as long as antenna in male, proportionately somewhat shorter in female.... **dasythrix**
   Hairs of thorax and abdomen shorter and more bristle-like; acrosticals stout, bristle-like; scutellum with two pairs of marginals and one of discals; clypeus or at least epistoma yellow, more or less obscured by grayish pollen; tibiae usually yellow to reddish-yellow, sometimes infuscated centrally, rarely entirely black; palpus distinctly clavate, entirely yellow, and shorter, not more than 1.2 as long as antenna, **impudicum**

[2]The convexity above the metathoracic spiracle and before the halter.

[3]The term "clypeus" is used here in the sense in which Townsend employes it, that is, as the middle of the "face," or as the "frontoclypeus" of Comstock.

7. Hypopleuron with a few long fine hairs on the upper margin in front of the metathoracic spiracle; presutural acrosticals sparse and bristle-like, biseriate; arista with hairs on basal half, the longest about twice as long as the maximum width of the arista ..... **frigidum**
   Hypopleuron bare 8
8. Anterior pair of postsutural intraalars lacking 9
   Anterior pair of postsutural intraalars present, the posterior pair, however, sometimes weak or lacking 10
9. Both pairs of postsutural intraalars lacking; presutural acrosticals minute, irregularly triseriate or quadriseriate; wings of normal length, pale yellow, the cross-veins not infuscated; antennae largely black; posterior portion of thorax between base of abdomen and bases of hind coxae as usual for the genus, that is, not evenly convex, the central portion less heavily sclerotized than the lateral portion and more or less sunken; male hypopygium enlarged, fifth sternum incised medially, with prominant lateral processes ..... **orcasae**
   Posterior pair of postsutural intraalars present; presutural acrosticals moderately strong, biseriate; wings exceptionally long, brownish-yellow, with the cross-veins infuscated; antennae wholly yellow; posterior portion of thorax between base of abdomen and bases of hind coxae convex and equally heavily sclerotized for entire width; male hypopygium of ordinary size, fifth sternum with paired median but without lateral processes ..... **reses**
10. Arista with some outstanding hairs on the basal half, the longest of which are not less than four times as long as the basal diameter, or fully one-third as long as the width of the third antennal segment 11
    Arista bare or pubescent, hairs when present not noticeably longer than the basal diameter 13
11. Third antennal segment almost entirely black; palpus on apical half very distinctly dilated, more than breadth of third antennal segment; cross-veins distinctly infuscated; legs fulvous-yellow, the front femur with a blackish streak posteriorly (*palpale* Malloch),
    female of **Pseudopogonota aldrichii** Malloch
    Antennae fulvous-yellow, the third segment sometimes slightly brownish above and at apex, never black; palpus clavate, not markedly dilated; legs entirely yellow 12
12. Occiput on upper half black or blackish, somewhat obscured by yellowish to golden pollen; fifth abdominal sternum of male in the middle of its apex with a pair of slender, closely-placed processes which are longer than wide ..... **suillum**
    Occiput yellow, at most somewhat darkened in the median area behind the ocellar triangle; fifth abdominal sternum of male in the middle of its apex with a pair of short, broad, rounded processes ..... **pallidum**
13. Femora deep black and conspicuously glossy, the tibiae and tarsi also black; fore tarsus very short, the fifth segment almost as long as the first and almost as long as the second to fourth segments combined; male usually with extremely long orange-yellow crinkly hair on abdomen; bristles and hairs on mesonotum long and strong, the presutural acrosticals biseriate and setulose or bristly; fifth abdominal sternum of male with its lateral angle produced into long tapering processes and without central processes ..... **crinitum**
    Femora never glossy black, if black, then more or less distinctly gray-pollinose, or the tibiae are not black; fore tarsus slender, the fifth segment much shorter than the first and hardly as long as the third and fourth combined; abdomen without dense crinkly hair in either sex; fifth abdominal sternum of male with slender median processes and without lateral processes 14
14. Inner cross-vein but little beyond apex of first vein; usually the fore femora partly blackish, the mid and hind pairs sometimes darkened on the posterodorsal surfaces; longest hairs on the arista not as long as its basal diameter ..... **furcatum**
    Inner cross-vein about as far beyond apex of first vein as length of the costal division immediately preceding the latter; all femora darkened

on their posterodorsal surface, the mid and hind pairs with a fuscous streak from near base to beyond middle; longest hairs on arista a little longer than its basal diameter..... **vittatum**

THE SCOPEUMA STERCORARIUM GROUP

Scopeuma stercorarium (Linnaeus)

Musca stercoraria Linnaeus, 1763, Fauna Suecica, second edit., p. 458, no. 1861. Type from Europe.

This species, the most common and widely distributed member of the genus, is the ubiquitous dung fly found so commonly on fresh cattle dung. It occurs throughout much of temperate North America, Europe, and Asia, and into Africa. Though highly variable in appearance because of the variation in the color, extent, length, and density of its yellowish vestiture, it is easy to distinguish from other members of the genus by the characters given in the key. I agree with Malloch that there is no basis for distinguishing *Musca merdaria* Fabricius, a less hairy form but one which shows all degrees of intergrades in the same localities as the more typical *S. stercorarium*.

A peculiar gynandromorph from Giant Forest, Tulare County, California, July 26, 1928 (C. L. Fox), Calif. Acad. Sci., is apparently a female with the postabdomen and genitalia showing male characters. The first four abdominal segments are broad, with the vestiture consisting of short, appressed, black setulae, as in a normal female. The remaining segments are predominantly male, but with female characteristics: the black setulae persist on the fifth and sixth terga though mixed with softer whitish pile; the fifth sternum is prolonged on one posterior angle only, and lacks the median processes; a well-developed though undersized, two-segmented hypopygium is present, both segments of which are shining, the first segment being reddish and the second black as in the corresponding segments of the female, and unlike the normal male condition; the second hypopygial segment is clothed on each side with converging black hairs and bristles; apparently normal, though small, claspers, and an aedeagus are present. A normal male and female, same data except July 18, are of the type corresponding to *Musca merdaria*.

Distribution.—I have records from all eleven Rocky Mountain and Pacific Coast States, from Alberta, British Columbia and Alaska. Farther northward, *S. furcatum* appears to replace it as the most common species.

THE SCOPEUMA MOLLE GROUP

The three species referred to this group are obviously closely related and seem to form a natural complex. The genitalia are of the same type in all three (cf. Malloch, 1935, p. 252, for male and female genitalia).

Scopeuma molle (Becker)

Scatophaga mollis Becker, 1894, Berliner Ent. Zeitschr., 39: 171. Type from Siberia.

This species is very close to *S. griseum*, the best distinguishing characteristic being the soft, long hairs on the convexity of the pleurotergite, above the metathoracic spiracle and before the halter. Crossvein r-m is usually distinctly clouded, and the chaetotaxy is stronger.

Specimens examined.—12 males and females. ALASKA: Matanuska, May 10, 1944, rotary trap (J. C. Chamberlin), U. S. Nat. Mus. COLORADO: Roggen, April 8, 1933, and May 18, 1946 (M. T. James), Colo. A. & M. Coll., and U. Colo. UTAH: Logan, Sept. 9, 1931 (Lowell Cutler), June 11, 1931 (Wylie Thomas) and Oct. 17, 1909; Blue Creek, Nov. 4, 1931 (G. F. Knowlton); all Utah State Ag. Coll.

Malloch has recorded this species from Northwest Territory, Yukon Territory, and Alberta.

Scopeuma griseum (Malloch)

Scatophaga grisea Malloch, 1920, Proc. Ent. Soc. Washington, 22: 34. Type locality, Logan, Utah.

A larger and more robust species than ***hiemale***, from which it may readily be separated by the key characters. The brownish markings of the thorax, especially on the mesonotum, are more slender and less conspicuous, and the abdominal pollen is unicolorous. The chaetotaxy is essentially as in ***hiemale***, though tending to be better developed. The front femur is a little shorter than in ***hiemale*** and tends to taper more apically.

Specimens examined, 163 males and females.—CALIFORNIA: Bishop, July 28, 1940, Lake Tahoe, July 11, 1940, and Lone Pine, July 28, 1940 (all L. J. Lipovsky), U. Kansas. COLORADO: Lindland, July 27, 1938, Cowdrey, July 27, 1938, Nederland, July 9, 1932, Grizzly Creek Camp Ground, Jackson County, July 17, 1946, and Rand, July 18, 1946 (M. T. James); LaVeta Pass, Aug. 5, 1938 (M. T. James, Urless Lanham), Colo. A. & M. Coll.; North Park, 9000 ft., July, Chicago Mus. N. H. IDAHO: Succor Creek, July 30, 1926 (R. W. Haegele) and Mackay, July 7, 1926, U. Idaho. MONTANA: Beaver Creek, 6300 ft., August, 1913 (S. J. Hunter), U. Kansas; Bennett, Aug. 12, 1931 (R. H. Beamer, J. Nottingham), U. Kansas. NEVADA: Wells (allotype); Wells, June 3, 1940 (M. T. James), Colo. A. & M. Coll.; Carson City, Aug. 9, 1929 (P. W. Oman), U. Kansas. CALIFORNIA: Blue Lake, Lassen County, July 12 to 20, 1947 (T. F. Leigh), U. Calif. OREGON: Anthony Lake, July 11, 1931 (R. H. Beamer and students), U. Kansas; North Powder, July 13, 1931 (J. Nottingham), U. Kansas; Dixie, July 8, 1931 (Beamer), U. Kansas; Haines, July 30, 1931 (L. D. Anderson), U. Kansas; Klamath Falls, May 8, 1924 (C. L. Fox), Calif. Acad. Sci. UTAH: Logan (type); Heber, Aug. 17, 1940 (Beamer), U. Kansas; Altus, June 26, 1940 (James), Colo. A. & M. Coll.; Logan, May 28, 1947 (M. Varzendeh) and Aug. 23, 1907 (E. G. Titus), Utah State Ag. Coll.; Logan, Aug. 15 and 18, 1922 (E. P. Van Duzee), Calif. Acad. Sci.; Logan Canyon, June 6, 1938 (W. P. Nye), Aug. 14, 1943 (G. F. Knowlton, D. R. Maddock), and Sept. 21, 1942 (Knowlton, S. L. Wood), Utah State Ag. Coll.; Ephraim, June 15, Utah State Ag. Coll.; Plain City, June 14, 1929 (Knowlton, R. S. Roberts), Utah State Ag. Coll.; Mt. Nebo, Aug. 14, 1943 (Knowlton, Maddock), Utah State Ag. Coll. WYOMING: Hunters Creek, Sept. 11, 1895, Lusk, Aug. 26, 1895, and Two-gwo-tee-a Pass, Sept. 12, 1895 (all Hough Collection) Chicago Mus. N. H.; Lusk, July, 1895, Horse Creek, August, 1895, and near Lander, 5000 to 8000 ft. (Roy Moodie), U. Kansas; Snowy Range, near Centennial, Aug. 15, 1938 (M. T. James), Colo. A. & M. Coll.

Scopeuma hiemale, new species

Male.—Background of head mostly black; frontalia, at least on anterior half, and an adjacent area of each parafacial opposite bases of antennae, reddish-yellow; parafacials, clypeus, and oral margin yellow; background obscured by dense grayish pollen which is lightest on the frontalia but which almost completely obscures the coloration of the clypeus and lower parafacials. Comparative head measurements of holotype: head width, 93; head height, to oral margin, front view, 60; width of vertex, 42, of front opposite lunule, 36, anterior ocellus to lunule, 26; lunule to oral margin, 35; distance between major vibrissae, 28; width of parafacial, 4, of parafrontal above lunule, 5, of parafrontal opposite anterior ocellus, 12; length of antenna 30, of arista, 35, of palpus, 30; palpal width at base, 2.5, before apex, 4.5. One pair each of ocellars, inner verticals, and outer verticals, all strong and clearly differentiated from adjacent setulae; postocellars short but distinct; one proclinate and two reclinate pairs of frontoorbitals; usually four pairs (sometimes a fifth) of convergent frontals; one pair of cruciate vibrissae, with a shorter vibrissa above and one below on each side, and with a group of short bristles adjacent; normally no bristles on cheeks. Occipitoorbitals extending a little below middle of eye; a few scattered bristles, arranged mainly in a row on each side, on upper half of occiput. All bristles of head black; a few scattered black setulae on ocellar triangle, occiput, and vertex; pile of cheeks and lower part of occiput silky whitish.

Antenna deep black; second segment with some black setulae and an outstanding, though short, black bristle; arista black, thickened on basal third, with a few short appressed black hairs on first and thickened part of second segment. Palpus wholly yellow or at most slightly infuscated apically, grayish-pollinose, spatulate, mostly whitish-haired, but with a few stiff black hairs at apex.

Thorax black, obscured by grayish pollen variegated with brownish as follows: two broad vittae just inside the dorsocentral rows; two of similar breadth inside the intraalars, interrupted at the suture and reaching neither the humerus nor the postalar callus; and a stripe of similar breadth extending across the lower part of the humerus, across the mesothoracic spiracle, behind which it is interrupted, and almost to the posterior margin of the mesopleuron; these are much broader and more prominent than similar areas in *S. griseum*. Stronger bristles arising from distinctly brownish rings. Acrosticals hair-like, in about four irregular rows. Normally one pair of postsutural intraalars (the first), the second sometimes feebly developed, two pairs of marginal scutellars, and no discoscutellars, though one pair of the latter may be feebly developed. Stigmatals and propleurals absent. Propleura hairy. Five or six fine whitish hairs on each pteropleuron. Bristles, acrostical hairs, and mesonotal setulae black; pleural pile soft, whitish. All pile and setulae short, longest on sternopleura, where they are one-half to two-thirds length of antennae.

Legs robust; front femur much thicker than middle and hind ones. Coxae, trochanters, and femora, except apices, black, rest of legs yellow, all with dense grayish pollen, especially on the black areas. Chaetotaxy

of legs variable, but the apparent normal condition is: middle femur with one posterodorsal preapical and one posterior preapical; hind femur with an irregular row of four to five anterodorsals; fore tibia with a long median dorsal, a posterodorsal row of four or five, and a median preapical posteroventral; middle tibia with an anterodorsal, a posterodorsal, and a posterior; hind tibia with two median and one preapical anterodorsals and two median and one preapical posterodorsals. Pile of tibiae, tarsi, and apices of femora stiff, black, that of coxae and femora, except apices of latter, soft, whitish; halteres and calypters, with their fringe, yellow; wings without clouding or slightly clouded on the crossveins, yellowish at base; veins yellowish basally, brownish apically; veins R_{4+5} and M_{1+2} somewhat convergent, then parallel at extreme apices.

Abdomen black, obscured by dense pollen which is mostly grayish but which is distinctly brown at the broad bases and apices of terga two, three, four, and five; pollen of apical segments tending to brownish apically. Pile of first and second terga, sides of other terga, and of sterna, soft, whitish, a little longer than on thorax but by no means dense or crinkly, longest on sides of second segment; terga otherwise with black setulae; second to sixth terga each with a row of weak marginal bristles. Fifth sternum deeply incised medially, the arms extending U-shaped; no median processes.

Length, 4–5 mm.

Female.—Essentially as in the male, except sexually. Bands of brownish pollen on abdomen not quite so broad as in male. Last tergum appearing blunt and rounded from dorsal aspect.

Holotype, male,—Cimarron River, 28 miles south of Walsh, Colorado, May 23, 1947 (M. T. James); State College of Washington Type Collection No. 170. *Allotype*, female.—East Carrizo Creek, Las Animas County, Colorado, May 23, 1947 (James). *Paratypes:* 14 males, 5 females, East Carrizo Creek, Colorado, May 23, 1947 (James); 1 male, 1 female, 28 miles south of Walsh, Colorado, May 23, 1947 (James); 5 males, 1 female, Roggen, Colorado, November 24, 1945 (James); 1 female, Roggen, Colorado, May 27, 1937 (M. & H. James); 1 male, Fort Collins, Colorado, May 3, 1946; State Coll. Wash. and Colo. A. & M. Coll.

THE SCOPEUMA LITOREUM GROUP

The close relationship of *S. dasythrix* and *S. impudicum* to the European *S. litoreum* (Fallén) is quite apparent, even to the point that some question may exist as to the specific distinctness of the three. *S. frigidum* and *S. crinitum*, two well-marked species, are being placed here provisionally, as they share with the *litoreum* complex such characters as the dense hairiness and crinkly pile, the long biseriate acrosticals, the strongly developed chaetotaxy, and the similar genitalia, as well as having likewise a littoral habitat.

Scopeuma dasythrix (Becker)

Scatophaga dasythrix Becker, 1894, Berliner Ent. Zeitschr., 39: 173. Type from Bering Strait.

As Malloch has pointed out, this species is very close to both *S. islandicum* (Becker) and the European *S. litoreum* (Fallén). Typically

it is the hairiest member of this complex of three species, the long, crinkly hair of the abdomen of the male being blackish above, yellow below, and appearing as a whole grayish to the naked eye. The female is hairy for that sex, with crinkly pile on the pleura, sterna, and legs. The male from Neskowin, Oregon, is much less hairy than the Alaskan males, in this respect, except for the abdominal terga, being more like the Alaskan females.

Specimens examined.—99 males and females. ALASKA: St. Paul Island, June 20, 1920, July 4, 1920, and July 13, 1917 (G. D. Hanna), April 13, 1913 (A. G. Whitney), and July to August, 1921 (A. Christofferson), Calif. Acad. Sci. and U. S. Nat. Mus.; St. George Island, June 4 and 16, 1914, and July 1, 1920 (Hanna), Calif. Acad. Sci. and U. S. Nat. Mus.; Atka Island, Aug. 1, 1907 (E. C. Van Dyke), Calif. Acad. Sci.; Seward, June 15, 1937 (C. B. Philip), State Coll. Wash.; Douglas, Aug. 2 and 9, 1901 (Elred Jenne), State Coll. Wash.; Amchitka Island, July 5 and 8, 1937, U. S. Nat. Mus.; Kodiak, 1935, Towns Coll., U. S. Nat. Mus. OREGON: Neskowin, Aug. 10 to 17, 1948 (M. T. James), State Coll. Wash.

Scopeuma impudicum (Reiche)

Anthomyia impudica Reiche, 1857, Bull. Soc. Ent. France, v, p. ix.
Scatophaga Islandica Becker, 1894, Berliner Ent. Zeitschr., 39: 175. Types from Iceland and Labrador.
Scopeuma impudicum (Reiche), Sack, *in* Lindner, 1937, Die Fliegen der Palaearctischen Region, 62a, p. 61 (synonymy).

I have seen four males and two females, Larrabee State Park, near Bellingham, WASHINGTON, July 17, 1949 (M. T. James); in addition to this I have records from Labrador, Nova Scotia, Quebec (Gaspe Bay and Bradore Bay), and Alaska. Malloch has reported this species from Churchill, Manitoba, and from the Pribiloff Islands.

Scopeuma frigidum (Coquillett)

Scatophaga frigida Coquillett, 1900, Proc. Washington Acad. Sci., 2: 454. Types from Kukak Bay and Popof Island, Alaska.

According to Malloch (1935), this species was, at the time he wrote, known only from the types (two males and one female, Kukak Bay and Popof Island, Alaska). I have seen a male, Adak, Aleutian Islands, ALASKA, Aug. 3, 1947 (D. W. Jenkins), U. S. Nat. Mus., and a female, Unalaska, ALASKA, Sept. 10, 1920 (G. D. Hanna), Calif. Acad. Sci., which conform with the original description. The fifth sternum is essentially as in *S. crinitum* (cf. Malloch, 1935 p. 259, fig. 18), but at least the lateral apical processes are yellow. I have seen two males, Newport, OREGON, June 8, 1925 (E. C. Van Dyke), Calif. Acad. Sci.; three males, Smith River, CALIFORNIA, July 12, 1930 (J. M. Aldrich), U. S. Nat. Mus.; one male and one female, Crescent City, CALIFORNIA, July 10, 1930 (Aldrich), U. S. Nat. Mus.; one female, Carmel, CALIFORNIA, Oct. 16, 1921 (L. S. Slevin), Calif. Acad. Sci.; and one male, one female, Hermosa Beach, CALIFORNIA, March 18, 1941 and May, 1938, U. Calif. These seem to be clearly conspecific with the Alaska specimens, but are paler; the coxae and tibiae tend to be more brightly

yellow, the femora tend to be broadly yellow basally and apically, though with some variation in this respect, the fifth sternum in the male may be almost entirely yellow, and the pale pile, particularly on the abdominal venter, is more brightly yellow. The length varies from 5 mm. (Crescent City) to 10.5 mm. (Newport), the Alaska specimens being intermediate.

Scopeuma crinitum (Coquillett)

Scatophaga crinita Coquillett, 1901, Proc. U. S. Nat. Mus. 23: 612. Type from Bering Island.

The glossy black femora and the shortened front tarsi distinctly characterize this species, although there is a tendency for the front tarsi to become shortened in some species, *S. frigidum* for example. The acrosticals are strong and bristle-like; there are usually four or five postsutural intraalars and five or six postsutural dorsocentrals, thus making the mesonotum conspicuously bristly. In typical males, the abdomen is covered with dense, furry, crinkly, yellow pile; this is the hairiest species discussed in this paper.

I have seen about two hundred specimens from coastal and island localities in Alaska. This species probably does not extend southward into our area.

THE SCOPEUMA ORCASAE GROUP

Scopeuma orcasae (Malloch)

Scatophaga orcasae Malloch, 1935, Ann. Mag. Nat. Hist. (10)15: 265.

Malloch states that this is an aberrant species, but that on the basis of the present definition it must go in this genus. The type and allotype are from Mt. Constitution, Orcas Island, Washington, July 7, 1905.

THE SCOPEUMA RESES GROUP

Scopeuma reses (Giglio-Tos.)

Scatophaga reses Giglio-Tos, 1893, Boll. R. Univ. Torino, 8: 158.
Scatophaga suilla var. *mexicana* Malloch, 1935, Ann. Mag. Nat. Hist. (10)15: 258, (new synonymy).

Malloch's holotype of *mexicana*, U. S. National Museum No. 50445, is simply a slightly teneral specimen of *reses*. In couplet 20 of his key, Malloch separates three species, namely *scybalaria* L., *reses* G.-T. and *amplipennis* Portschinsky from the species of the following couplets, including *suilla* s.s. and *suilla* var. *mexicana*, on the basis of the convexity and heavy sclerotization of that part of the thorax lying between the abdomen and the hind coxae. In the type of *mexicana*, this portion is damaged, but is apparently concave; this concavity, however, as well as the lack of clearly defined dark tergal apices, seems to be due to the teneral condition of the specimen. Contrary to Malloch's statement, the posterior intraalar bristle is present.

Specimens examined.—62 males and females. NEW MEXICO: Clouderoft, June 27, 1940 (R. H. Beamer, D. E. Hardy), Magdalena, and from Magdalena Mts., Aug. 1894 (Snow), U. Kansas. ARIZONA:

Chiricahua Mts., June 9, 1933 (Beamer), U. Kansas. MEXICO: Mt. Tancitaro, Michoacan, 1941, 7800 ft., (H. Hoogstraal), Chicago Mus. N. H.; 43 km. S. of Mexico, D. F., 9480 ft., June 20, 1948 (W. Nutting), State Coll. Wash.

This species has been recorded from Guatamela by Malloch.

THE SCOPEUMA SUILLUM GROUP

This is a group of slender species with slender legs, not especially conspicuously hairy bodies, and a similar type of male genitalia. The fifth sternum bears a pair of approximate slender or rounded median processes, but is without lateral processes.

Scopeuma suillum (Fabricius)

Musca suilla Fabricius, 1794, Entomologia Systematica, vol. 4, p. 343. Type from Europe.

This Holarctic species seems to be widespread, though not common, in the northern part of our range, and thence northward to Alaska.

Material examined.—About 35 males and females. BRITISH COLUMBIA: Merritt, U. Kansas. WASHINGTON: Ilwaco, May 16, 1918 (A. Spuler), State Coll. Wash; Quinault and Republic, U. Kansas. OREGON: Neskowin, Aug. 10-17, 1948 (James), State Coll. Wash. MONTANA: Missoula, U. Kansas. UTAH: Logan Canyon, Sept. 16, 1934 (T. O. Thatcher), Utah State Coll.; Hole-in-Rock Canyon, Uinta Mts., Aug. 16, 1940 (D. G. Hall), U. S. Nat. Mus. COLORADO: Science Lodge, near Ward, June 17–19, 1939 (Urless Lanham) and May 9, 1939 (H. G. Rodeck, Urless Lanham), U. Colo.; Lindland, July 27, 1938 (James), Masonville, Sept. 3, 1934, Ward, July 14, 1939 (James), and Grizzly Creek Camp Ground, Jackson County, July 17, 1946 (James), all Colo. A. & M, Coll.; Walden, Pingree Park, and Sloss, all U. Kansas; Cameron Pass, Aug. 23, 1940 (D. G. Hall) U. S. Nat. Mus.

Malloch (1935) and Strickland (1938) have recorded this species from Alberta.

Scopeuma pallidum (Walker)

Scatophaga pallida Walker, 1849, List of specimens of Dipterous insects in the collection of the British Museum. Part iv, p. 981. Type, Martin Falls, Canada.

This species is probably extralimital. It is of relatively common occurrence in Southeastern Canada and the Northeastern United States, at least as far south as North Carolina. Malloch says it probably occurs chiefly in the mountains.

Scopeuma furcatum (Say)

Pyropa furcata Say, 1823, Jour. Acad. Sci. Philadelphia, 3: 98. Type from Missouri.

Scatophaga squalida Meigen, 1826, Systematische Beschreibung der bekannten europäischen zweiflügeligen Insekten, vol. 5, p. 252.

Though in recent publications European authors (Collin, Carpenter, Sack) have recognized the above synonymy, they have tended to overlook the fact that *furcata* Say has three years' priority over *squalida* Meigen.

This species, like several others in the genus, is quite variable. Malloch in his table of species, states that "only the fore femora [are] partly fuscous, the mid and hind pairs not darkened on the posterodorsal surfaces." However, in an occasional specimen the mid and hind femora are slightly darkened, though for the most part yellow. The "fuscous" marking of the front femora I should characterize as blackish, though this color is considerably dimmed by the overlying pale pollen. An occasional specimen has the front femora wholly yellow.

Widely distributed throughout the Holarctic region. I have numerous records from BRITISH COLUMBIA, ALBERTA, WASHINGTON, OREGON, CALIFORNIA, IDAHO, MONTANA, WYOMING, UTAH, COLORADO, and NEW MEXICO. It is more common farther northward in its range and at higher elevations, where it may replace *S. stercorarium* as the dominant species.

Scopeuma vittatum (van der Wulp)

Scatophaga vittata van der Wulp, 1897, Biologia Centrali-Americana, Vol. 2, p. 350. Type from Mexico City.

This species is probably extralimital. It is known only from Mexico.

SPECIES ERRONEOUSLY REFERRED TO SCOPEUMA

Pseudopogonota aldrichii Malloch

Pseudopogonota aldrichii Malloch, 1920, Proc. Ent. Soc. Washington, 22: 35.
Scatophaga palpalis Malloch, 1931, Ann. Mag. Nat. Hist. (10)8: 443 (new synonymy).

The above synonymy seems virtually certain. Malloch uses the "spoon-shaped" palpi to distinguish *Pseudopogonota* from *Scatophaga*, but in his description of *S. palpalis*, he used this same character ("palpi . . . very distinctly widened apically") to separate his species from *suilla*. *P. aldrichii* was described from both males and females; it is odd that Malloch failed to recognize the female when he described *S. palpalis*. The males of *P. alrichii* are easily recognizable by their genitalia and associated structures; this easily explains why the males of *S. palpalis*, as such, have never been recorded.

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UTILIZATION OF FOOD BY SINEA DIADEMA (FABR.) (Reduviidae, Hemiptera)[1]

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INTRODUCTION

During the past ten years I have published a series of articles on several bionomic features of two predatory entomophagous Hemiptera, *Sinea diadema* and the phymatid, *Phymata pennsylvanica americana* Melin. A list of these papers is appended for the convenience of interested persons. Among the more recent of these are three that pertain to (a) weight patterns and (b) variability in the duration of the insects. In the course of the work on these two problems, there accumulated a large body of data which now prove to bear also on the utilization of food by the nymphal instars of the above predators. The data relative to *Sinea* are better suited to this question than are those from *Phymata*, and for this reason the present article deals exclusively with *Sinea*. The reader will recognize that the plan of the project on weights and instars did not envisage the results on food-use presented below, and that these are therefore in the nature of by-products. The procedure might have been better designed than it was, had utilization of food been among the original objectives of the undertaking. For example, the diets administered to the several series of experimental bugs were not evenly graduated as to amount, hence the results do not lend themselves to expression in the form of a curve. Yet the data are, I believe, of such significance with reference to insect nutrition as to warrant this publication.

The materials below relate to two features pertinent to research on insect nutrition; first, description of a method of calculating the approximate weight of the food utilized by a predatory insect equipped with fore legs for seizing prey and mouthparts for removing liquids or liquified solids from the prey, leaving the empty chitinous cuticle to be discarded; and second, analysis of some data obtained from *S. diadema* by this method.

METHOD OF DETERMINING THE WEIGHT OF FOOD UTILIZED

Investigators contemplating a study on the quantity of food used by a sucking predator would probably first consider the method whereby the prey is weighed immediately before it is offered to the predator, and again at once after the indigestible chitinous remains of the prey are discarded, the difference between the two weights being the bulk of substance ingested. However, application of this direct method would promptly show it is impractical for the following reasons: (1)

[1]Contribution No. 287 from the Entomological Laboratories of the University of Illinois. This study was aided by an appropriation from the Research Board of the Graduate School. I am pleased to acknowledge the critical reading of the manuscript by my colleagues, Doctors G. S. Fraenkel and William R. Horsfall.

The sucking predator must be supplied throughout its nymphal life of several weeks with prey of a size from which all the digestible, non-chitinous contents may be removed in one continuous feeding operation; (2) the predator utilizes large numbers of such prey individuals while growing up; (3) it cannot be relied on to feed at once when the prey is placed in the cage, with the consequence that the latter loses significant weight from activity and lack of food, and (4) this direct procedure requires alert attention from a group of observers every moment of the nymphal life in order to secure accurate weights of the prey at once preceding and following the feeding of the individual predators employed in the experiment. In short, the direct approach to the problem of nutrition wherein a sucking predator is concerned is obviously not feasible.

For the above reasons, the indirect method, described below, is more desirable for approximating the amount of food which *S. diadema* may ingest. The prey employed in securing such results consisted exclusively of adult flies of *Drosophila melanogaster* (L.). While the method gave fair indications of the quantity of food withdrawn by nymphs of the third and fifth instars, and less precise indications for the first and second, it proved a relatively satisfactory measure for the fourth instar for the reasons that nymphs of the latter stage readily capture the *Drosophila* and thus secure that quantity of food necessary for approximately optimal growth, and they have the capacity to withdraw all the ingestible or liquefiable substance from the flies secured, leaving them light and transparent. By their light weight and transparency, the discarded flies may be known to have been emptied of their digestible contents. As a consequence of these reasons, I shall emphasize the data for the fourth instar. While therefore compelled to employ estimates rather freely in calculating the quantity of food ingested by the first two instars, this necessity incurs no serious error because the amount of food utilized by them constitutes a small part of the total required in the entire nymphal life. The actual weights are employed where the third, fourth and fifth instars are concerned.

The method of calculating the amount of food used by any individual, either during any one stadium or in the entire nymphal life, employs a single value, namely the average amount, by weight, of ingestible substance contained in individual adults of *D. melanogaster*. The procedure in obtaining this value was (1) to weigh 4649 normal living flies in lots of 10 to several hundred each, these being inactivated, for the purpose of weighing, by refrigeration at once upon removal from the rearing tubes; (2) to weigh more than 9000 flies from which all or most of the ingestible liquefiable substance was removed by *Sinea*, such discarded emptied flies being handled in numerical lots of sizes that permitted accurate weights, and as they become available daily from the cages housing the predator. The actual average weight of single flies discarded by *Sinea* was 0.000067043 gram, and the average weight of the single whole inactivated living flies was 0.000831404 gram. The difference between these figures is 0.000764361 gram, which is taken here to be the average quantity of substance removed by suction from individual *Drosophila*. In the course of securing the data, each individual of *Sinea* was kept by itself in a shell vial; and the number

of flies utilized by each bug from day to day was recorded. The number of discarded cuticles was multiplied by the value 0.0007643 in order to obtain the approximate weight of food ingested by an individual nymph of *Sinea* either in any one of the five instars, or in the entire nymphal life. For the sake of making the weights derived by this method more easily comprehended, they are converted into milligrams below.

TABULATED DATA AND METHOD OF CALCULATION

It should be understood that some of the individual *Sinea* in the experimental series failed to complete their nymphal development either

TABLE I

RELATION BETWEEN THE AMOUNT OF FOOD INGESTED AND THE AMOUNT ASSIMILATED BY NYMPHS DURING THE FOURTH INSTAR

AMOUNT INGESTED PER NYMPH	NYMPHS FED	FLIES CONSUMED PER NYMPH	NET GAIN IN WEIGHT	AMOUNT ASSIMILATED
Milligrams	*Number*	*Number*	*Milligrams*	*Percent*
		MALES		
0.0– 9.9	4	10–12	2.3– 3.3	25–43
10.0–19.9	2	14–20	2.9– 4.3	27–28
20.0–29.9	6	27–39	4.0– 7.8	20–27
30.0–39.9	11	42–52	7.0– 9.5	19–29
40.0–49.9	12	53–65	7.9–10.8	18–25
60.0–69.9	2	79–83	8.0–10.5	13–16
		FEMALES		
0.0– 9.9	1	13	4.0	40
20.0–29.9	3	27–31	5.3– 7.4	22–36
30.0–39.9	9	44–52	5.9–10.2	17–28
40.0–49.9	9	53–65	8.4–13.1	19–28
50.0–59.9	3	66–74	8.6–11.8	15–23

because of accidents in handling, or more frequently because of starvation. Considered below are only those individuals which did survive the dietaries and became adults. It will be noted also that the records for the two sexes are tabulated separately; this is done for the reason that females usually feed more voraciously and ingest a larger bulk of food than the males. Table I contains data that relate to the fourth instar of the males and females, respectively, while Table II pertains to the sum of the five instars, *i.e.* the whole nymphal life. The records are tabulated in the order of the milligrams of food ingested by the several nymphs, and classified or grouped on the basis of quantitatively similar diets utilized (first column).

While the rather extreme range in number of flies used by the feeding bugs is largely the result of my intentional regulation of the amount of the food administered, it probably reflects also some inherent

differences in the feeding proclivities of the individuals. The tables further present data pertinent to (2) the number of nymphs forming each group, (3) the number of *Drosophila* whose digestible contents were withdrawn by the nymphs, (4) the net gain in weight achieved by each group of nymphs, and (5) the percent of the ingested substance that was assimilated as contrasted with the percent eliminated as feces,

TABLE II

RELATION BETWEEN THE AMOUNT OF FOOD INGESTED AND THE AMOUNT ASSIMILATED DURING THE WHOLE NYMPHAL LIFE

AMOUNT INGESTED PER NYMPH	NYMPHS FED	FLIES CONSUMED PER NYMPH	NET GAIN IN WEIGHT	AMOUNT ASSIMILATED
Milligrams	*Number*	*Number*	*Milligrams*	*Percent*
		MALES		
30.0– 39.9	1	49	14.0	38
40.0– 49.9	4	54– 64	9.1–17.7	22–36
50.0– 59.9	3	67– 76	12.6–14.5	22–28
60.0– 69.9	3	81– 89	14.1–15.4	22–23
70.0– 79.9	1	95	17.2	24
110.0–119.9	3	145–155	22.9–30.5	19–28
120.0–129.9	5	161–170	20.7–33.6	16–27
130.0–139.9	6	173–182	21.5–31.5	17–24
140.0–149.9	5	186–193	28.4–31.1	20–22
150.0–159.9	3	197–204	31.1–37.8	21–24
160.0–169.9	3	211–219	31.4–36.0	18–22
170.0–179.9	2	233–235	33.7–38.3	19–21
180.0–189.9	1	239	35.8	21
190.0–199.9	1	260	35.6	18
210.0–219.9	1	284	36.8	17
		FEMALES		
40.0–49.9	1	61	17.0	36
60.0–69.9	1	83	16.0	25
80.0–89.9	1	106	18.1	22
120.0–129.9	2	161–164	27.7–29.3	23
130.0–139.9	2	177–181	41.1–43.6	30–32
140.0–149.9	1	189	36.3	25
150.0–159.9	6	197–207	38.0–41.4	25–27
160.0–169.9	2	211–213	38.5–38.8	19–24
170.0–179.9	1	226	42.0	24
220.0–229.9	1	293	38.9	17.4
230.0–239.9	1	305	46.9	20.1

gases and exuviae. The net gain was obtained by subtracting the weight of the individual on the first day from its weight on the last day of any instar, or the total nymphal life. The percents of material (1) converted into bodily substance and (2) eliminated as wastes were then calculated by dividing the weight of the ingested matter into the net weight gained during the instar or total nymphal life, and multiplying the result by 100.

DISCUSSION

Following are the principal results obtained by the above-described method of study with reference to the utilization of food by *Sinea diadema*.

The amount, by weight, of food adequate to bring individuals through the fourth instar varied, in the series of males, from 7.6 to 63.4 mg., and in the series of females from 9.9 to 68.7 mg. The weight of food utilized by individuals during the entire nymphal life of five instars ranged, in the series of males, from 37.4 to 217.1 mg., and, in the series of females, from 46.6 to 233.1 mg. In terms of numbers of adult *Drosophila*, the male individuals imbibed the digestible contents of 10 to 83 flies, and the females 13 to 90 flies while undergoing the fourth instar; and growth through the whole nymphal life required from 49 to 284 flies, and from 61 to 305 flies, in the males and females, respectively. The extreme ranges of variability with reference to the quantity of food utilized in the four cases cited are in the ratio of 8.0, 7.0, 5.8 and 5.0 to 1, respectively. The resulting adults varied likewise with reference to weight, vigor, length of life and reproductive capacity, the values of these functions being somewhat directly proportionate to the amount of the diet allowed as indicated in the tables. The females allowed the smaller numbers of flies produced and laid no eggs, while those fed at an approximately optimal rate laid eggs of normal size, color and form, and in numbers adequate to promulgate the species, although not to the maximum extent. Specifically, a female that ingested 222.4 mg. during its nymphal and adult lives, produced only one egg, obtained by postmortem dissection; whereas the female that consumed 370.6 mg., as nymph and adult, laid 39 eggs. Amounts less than 222 mg. were insufficient for the genesis of eggs.

A second feature of note that was revealed in this study is indicated in the fourth and fifth columns of the tables. It pertains to the relation between the amount of food ingested and the percent of the food that was assimilated from the digestive tract into the blood and converted into more or less permanent body substance. Examination of these data show that those *Sinea* which received the smallest amounts of food by weight, both in the fourth instar alone and in the entire nymphal life, made the most efficient use of the material ingested. Comparison of the data from top to bottom in the two columns reveals that the percent of food assimilated gradually decreases as the amount of the diet is increased. Specifically, the male that developed to adult on the least bulk of food, *i.e.* 37.4 mg., or 49 flies, assimilated 37.65 percent of the material ingested, while expending 62.35 percent as wastes. By contrast, the well fed male that developed on 217.1 mg. of food, or 284 flies, assimilated only 16.95 percent, while eliminating 83.05 percent. In general, the individuals falling between the two extremes cited, assimilated less and less while expending more and more of the ingested substance as wastes. However, the rate of growth, as indicated by the data on net gain in weight, increased with each enlargement of the diet, although the rate of acceleration in growth appears to decline somewhat as the diet approached the maximum in amounts allowed.

The above data indicate the principle that assimilation is inversely

proportional to the amount of food ingested, while elimination, particularly of fecal wastes, is directly proportional to the quantity of the food ingested. As a possible explanation of this reversal in effectiveness in the use of food, I venture the thought that this phenomen is, at least in part, the result of variation in mechanical pressure of food in the alimentary tract. The smaller bulk ingested by individuals that are allowed a quantitatively low diet, passes slowly through the tract and is thus exposed a longer time to the absorptive surfaces of the midgut, with the result that a larger percent of the material becomes assimilated and a smaller part remains to be eliminated as feces. On the other hand, the larger to large bulks of food ingested through more frequent and more voluminous feeding by individuals allowed all the food they were able to take, enable the gut walls to force the material through the tract in a shorter time, with the consequence that relatively less of the nutrient elements can be assimilated or utilized.

CRITICISM OF THE METHOD

This indirect method of determining the amount and disposition of the food used by *Sinea* obviously has its shortcomings. In reflecting upon my experience with it, I can now make suggestions that may reduce the amount of error incurred. First, the predatory bugs, weighed individually and daily in my work, might be weighed hourly; this would be desirable especially during the molting phase of each instar. Second, the average weight of a living *Drosophila* could perhaps be determined more precisely if the flies were inactivated, for the purpose of facilitating their weighing, by means of a gas, because refrigeration probably causes condensation of moisture on the cold bodies after transfer to the balance and thus prejudices the results. Third, all flies discarded by the predator should be examined with a binocular in order to estimate more accurately the amount of ingestible substance that may have been left in them. However, in the large majority of cases, practically 100 percent of the substance is removed. Fourth, there was doubtlessly some disparity between the theoretical weight of the substance removable from an individual *Drosophila* and the weight of the substance actually contained in flies provided for the predator: for instance, while one individual *Sinea* probably chanced to receive mostly smaller flies during the nymphal life, another chanced to receive larger flies, with the result that some bugs were possibly smaller or larger, and yet are recorded in the tables as having utilized about equal numbers of flies. This shortcoming might be minimized if the experimenter would use only *Drosophila* produced during any one age period of the culture, *e.g.* the somewhat uniformly larger flies developing in the first days the culture is productive. This would require a larger number of cultures, but the number might be reduced if fewer bugs were included in the experiment at any one time. Were this suggestion adopted, a new average weight per fly would need to be established, based on the larger flies only, not on the entire yield of a set of cultures.

SUMMARY

A method of determining the approximate weight of food ingested

and assimilated by a predatory sucking entomophagous bug, *Sinea diadema*, is described.

Two general results obtained by this method are stated. First, the amount of food required to grow adults varied extremely, males having been produced on quantities ranging from 37.4 to 217.1 mg. of substance taken from adult *Drosophila melanogaster*, and females on amounts of 46.6 to 233.1 mg. The adults thus reared varied correspondingly in respect to their weight, rate of growth, vigor and reproductive capacity.

Second, individuals given a quantitatively small diet assimilated a higher percent of the food ingested than did individuals allowed a near-optimal diet. This inverse ratio of assimilation to amount of food ingested possibly results in part from differences in peristaltic action on small as compared with large bulks of food, the larger bulks passing through the tract in less time, with the consequent assimilation of a relatively smaller percent of the ingested substance.

Several suggestions are offered to improve the accuracy of results from the use of this method, should other experimenters desire to employ it in research on the utilization of food by sucking predatory bugs.

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SELECTED INVERTEBRATE TYPES, by F. A. Brown (Editor). xx+597 pages, 235 figures. John Wiley and Sons. 1950. Price, $6.00.

Thirteen contributors have collaborated to prepare a laboratory guide to the anatomy and physiology of a hundred common invertebrate types. Only two insects are included, *Periplaneta americana* and the larva of *Drosophila melanogaster;* but these parts should prove very useful to the entomologist. Other arthropods included are the horse-shoe crab, *Xiphosura polyphemus;* the spider, *Argiope aurantia;* the brine shrimp, *Artemia; Daphnia magna; Cyclops;* the goose-neck barnacle, *Lepas anatifera;* the mysid, *Heteromysis formosa;* crayfishes (and *Homarus*); the crab, *Callinectes sapidus;* and the millipede, *Spirobolus marginatus*. The authors of the parts dealing with the Arthropoda are J. H. Lochhead, J. B. Buck, and M. L. Keister.—M. T. J.

THE CHANGES IN THE THIAMINE, RIBOFLAVIN, NIACIN AND PANTOTHENIC ACID CONTENT IN THE FOOD OF FEMALE HONEYBEES DURING GROWTH WITH A NOTE ON THE VITAMIN K ACTIVITY OF ROYAL JELLY AND BEEBREAD[1]

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The term "royal jelly" refers to the product formed and secreted by the pharyngeal glands of the nurse bees for the nourishment of the queen larvae. Larvae which are to become the sexually underdeveloped female worker bees receive during the first two to three days of their development the "larval food" similar to that given the queen larvae. Afterwards they are believed to be supplied with the "larval food" which is poorer in nitrogenous materials and lacks a factor or factors necessary for the production of queens. Recent investigations (Haydak, 1943) showed, however, that the food of older worker larvae does not differ appreciably in its protein content from the food of older queen larvae, but is considerably lower in the fat and mineral contents. The major purpose of this study was to determine whether there are any differences in the concentration of certain of the B vitamins in the food supplied to the queen and worker larvae during the period of their growth.

The vitamin content of royal jelly has been determined by several workers. A review of the earlier studies has been given in previous papers (Haydak and Palmer, 1938, 1940, 1942). Recently Pearson and Burgin (1941) found royal jelly to be the richest source of pantothenic acid, containing 183 micrograms and 511 micrograms of this vitamin per gram of fresh and dry matter, respectively. Cheldelin and Williams (1942) made quantitative determinations of most of the known B vitamins in royal jelly. Their results are presented in Table I.

The results of the B vitamins determination in royal jelly made by Kitzes, Schuette and Elvehjem (1943) are in the same range with those of Cheldelin and Williams, the values being 320, 28, 111, 18, 10, 4.1 and 0.5 micrograms per gram for pantothenic acid, riboflavin, niacin, thiamine, pyridoxin, biotin and folic acid respectively. Kocher (1942), using the *Phycomycetes* test found 4.5–5.2 micrograms of thiamine per gram of royal jelly.

[1]Paper No. 2466, Scientific Journal Series, Minnesota Agricultural Experiment Station. Some of these studies were carried out in cooperation with the late Dr. L. S. Palmer (deceased 1944). Dr. O. Mickelsen of the Laboratory of Physiological Hygiene and Mrs. D. M. Sellers of the Division of Agricultural Biochemistry kindly carried out the determinations for thiamine and for niacin, riboflavin and pantothenic acid, respectively, in 1947.

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During the swarming season of 1942, royal jelly was collected from the cells containing queen larvae. The food was removed from the cells and kept separately, according to the age of the larvae. In this way the secretion was taken from open queen cells containing 1-, 2-, 3-, 4-, 5-day old larvae and from sealed cells with 6 to 7-day old larvae. It was found difficult to secure enough material for each of the consecutive days of this period in the case of worker larvae because of the minute quantities deposited in their cells. Therefore the food from the cells containing worker larvae from hatching to two days of age was combined, and the food from the cells containing larvae three to five days old also formed a single sample. The methods of analysis used were: for thiamine, that of Hennessy and Ceresedo (1939); for riboflavin the method of Snell and Strong (1939); for pantothenic acid that of Strong, Feeney and Earle (1941), and for niacin the method of Melnick and Field (1940). The antihemorrhagic activity of combined samples of royal jelly was determined by a slightly modified method of Almquist (1941).

TABLE I

B Vitamins of Royal Jelly (Micrograms per Gram)

(Cheldelin & Williams)

	Thiamine	Riboflavin	Pyridoxin	Niacin	Pantothenic acid	Biotin	Inositol	Folic acid
Fresh	6.6	8.2	2.4	59	89	1.7	100	0.20
Dry	21.0	26.0	7.7	190	290	5.4	310	0.62
Max.-Min. (fresh)	7.4-5.6	10.0-6.6	2.5-2.2	47-73	110-65	1.8-1.6	150-78	0.22-0.16

From the results of the analysis presented in Table II it is evident that there was not much change in the thiamine content of both fresh royal jelly and larval food given to the larvae during their growth period. However, when we compare the results on the dry basis then there was a slight variation in the food of the queen larvae and approximately a 30% drop in the content of thiamine in the food given to older worker larvae.

The thiamine content given in Table II is considerably below the values given in the literature. Haydak and Palmer (1940), using the rat assay method, found the thiamine content of royal jelly of 1939 to be equal to 3.1 micrograms/gm. of fresh and 9.1 micrograms/gm. of dry matter. Later investigations by the same authors (1942), using the microchemical method, showed the thiamine content of fresh royal jelly collected in 1940 to be 2.9 micrograms/gm. and for that collected in 1941 to be 1.5 micrograms/gm. Analysis by Haydak, using the microchemical method, of the fresh royal jelly collected in 1943, showed the values of 6.4, 3.9, 4.6, 4.7, 4.6 micrograms/gm. for the food taken from the cells containing 1-, 2-, 3-, 4-, and 5-day old queen larvae, respectively. Cheldelin and Williams (1942) and Kitzes and co-workers (1943) gave still higher values. It appears that the thiamine content

of royal jelly is quite variable, depending probably on the composition of pollen consumed by the nurse bees.

Determinations of the thiamine content of the fresh larval food collected in 1947, made at the University of Minnesota Laboratory of Physiological Hygiene, showed average values of 3.2 micrograms/gm. in the food of 1- to 2-day old worker larvae and 3.3 micrograms/gm. in that of 3- to 10-day old.

There was a decrease in the riboflavin content in the food given to the older queen and worker larvae. The fresh food of 2-day old queen

TABLE II

VITAMIN CONTENT OF LARVAL FOOD (MICROGRAMS PER GRAM)

Age of Larvae	Moisture	Dry Matter	Fresh Basis				Dry Basis			
			Thiamine	Riboflavin	Niacin	Pantothenic Acid	Thiamine	Riboflavin	Niacin	Pantothenic Acid
Queen. *Royal Jelly*										
1	65.37	34.63	1.3	8.3	149	200	3.6	24.0	430	578
2	69.17	30.83	1.2	6.1	101	180	4.0	19.8	327	583
3	69.88	30.12	1.2	5.9	91	194	4.0	19.7	302	644
4	69.70	30.30	1.2	5.6	91	199	3.9	18.6	300	657
5	67.58	32.42	1.2	5.3	99	172	3.6	19.6	305	530
Sealed *Royal Jelly* (combined results)	68.32	31.68	1.2	7.0	98	160	3.8	22.1	310	506
1-2	66.77	32.73	1.2	7.2	125	190	3.8	21.9	379	581
3-5	69.05	30.95	1.2	6.0	94	188	3.9	19.3	302	610
Worker. *Larval food*										
1-2	73.51	26.49	1.3	11.7	100	147	4.9	44.1	377	554
3-5	64.90	35.10	1.2	10.8	52	20	3.4	30.8	148	57
Pollen (Vivino and Palmer, 1944)			9.3	18.5	200	27.6	11.8	23.5	254	35.1
Worker larvae (1- to 3-day old) (Haydak & Vivino, 1943)			4.6	32.2	77.2	180.2	29.9	161.3	393.6	916.1

larvae had 26% less riboflavin than that of 1-day old. The content of riboflavin gradually diminished afterwards. On the dry matter basis the decrease was 17.5% on the second day.

An 8% drop in the riboflavin content of food given to older worker larvae was also observed. On the dry matter basis this drop is much more significant, amounting to 30% of that in the food of 1- to 2-day old larvae.

The decrease in the niacin content of royal jelly given to 2-day old queen larvae was 33% on the fresh and 24% on the dry matter basis. A considerably larger drop was observed in the niacin content of the

food of older worker larvae amounting to 48% on the basis of fresh and 61% on the basis of dry matter.

There was a slight decrease in the pantothenic acid content in the fresh food of older queen larvae. On the dry matter basis, however, there was a gradual increase in the panthothenic acid content of the food of older queen larvae up to the fourth day of their development, amounting to 14%. Then it dropped considerably (19%).

The pantothenic acid content of the food of older worker larvae abruptly diminished to a very great extent in comparison with the food of younger larvae, amounting to 86% and 90% for the fresh and dry weight of the food respectively.

In order to make a better comparison between the changes in the vitamin content of the food given to the queen and worker larvae during their development the results of analysis of the food of the queen larvae were grouped in the same way as in the case of worker larvae. Because of a considerable difference in the moisture content and a different trend in moisture changes (which increased in the food of older queen larvae by 3% and decreased considerably in the food of older worker larvae by 11.7%,) only a comparison on the dry matter basis is here made.

In spite of this grouping, it is somewhat difficult to compare the food of the queen and worker larvae of the same age, because the former were supplied with an overabundance of food which was carried over from one day to another, while worker larvae received considerably smaller daily doses and in the later part of their larval life the feeding was of a progressive type, the food being given as needed. Nevertheless, such a comparison is of interest. There is a slight increase in the thiamine and pantothenic acid content (by 5%) of the food of older queen larvae and a drop in the content of riboflavin (by 12%) and niacin (by 20%). However, as it has been pointed previously, there was a very sharp drop in the content of all vitamins investigated in the case of worker larvae, being equal to 30% in the content of thiamine and riboflavin and an especially large drop in the niacin (61%) and pantothenic acid (90%) contents.

Apparently the pharyngeal glands of the nurse bees do not have an ability to concentrate thiamine, riboflavin and niacin which they ingest with pollen, the latter having 9.3, 18.5, and 200 micrograms per gram of fresh matter respectively (Vivino and Palmer, 1944). On the other hand, the ability of the glands to concentrate pantothenic acid is amazing, because the pantothenic acid content of the dry matter of royal jelly is 16 to 19 times greater than that of pollen.

Young worker larvae also have an ability to concentrate thiamine, riboflavin and pantothenic acid, those values being 4.6, 32.2, and 180 micrograms per gram of fresh matter of their bodies and 29.9, 161.3 and 916.1 micrograms per gram of the dry matter respectively (Haydak and Vivino, 1943). These values are considerably large than those found for the larval food of workers.

It is evident from Table II that the concentration of riboflavin, niacin and especially pantothenic acid is very significantly lower in the food of older worker larvae. One is hardly justified in assuming that the nurse bees deliberately change the composition of their glandular secretion when feeding older larvae. However, Kohler (1922) and

Nelson and Sturtevant (1924) observed that the nurse bees add pollen to the food of older larvae. Planta (1888), to the contrary, found no pollen in the food of either younger or the older worker larvae. Microscopical examinations of our samples in 1942 showed an average of 6 grains of pollen and in 1948 only occasional grains of pollen per unit of a field of vision for the food of 1- to 2-day old larvae and an average of 11 and 9 grains for the same surface of the food of older larvae for years 1942 and 1948 respectively. The color and consistency of the food of both groups of larvae was also different. While the food of the younger larvae was milky white, showing a large number of white amorphous bodies present under microscope, the food of 3– to 5-day old worker larvae was grayish with a very few amorphous bodies present.

It is not likely that the presence of pollen (which is much lower in the pantothenic acid content than the food of younger larvae) in the food of older larvae is the cause of the lower pantothenic acid content of this food. If this were the case, then the thiamine, riboflavin and niacin content of the food of older larvae should have increased, because the amount of these vitamins in pollen is greater than that in the fresh food of younger larvae. Of course, the vitamin content of pollens varies and the nurse bees may consume beebread of various origin and age present in the hive. However, the drop in the vitamin content of the food of older larvae is a normal phenomenon, because a later (1948) check on the vitamin content of the food of younger and older worker larvae showed that the riboflavin, niacin and pantothenic acid content of the fresh food has dropped from 11.7, 81.7, and 128.6 micrograms per gram in the fresh food of 1- to 2-day old larvae to 10.8, 45.3 and 19.5 micrograms per gram in the food of older larvae respectively.

According to Rösch (1925), younger larvae, under normal conditions, are fed by bees 6 to 10 days old, which have reached their highest development (Haydak, 1934), while the younger, 3- to 5-day old bees, supply the food to the older larvae. It seems probable that the change in the concentration of the vitamins in the food of older larvae is due to the fact that the pharyngeal glands of the young bees are not sufficiently developed to secrete the larval food having the same vitamin content as that secreted by the older nurse bees. It is also conceivable that because the younger bees continue to grow up to the fifth day of their lives, the requirement for vitamins, especially those associated with intracellular respiratory enzymes, is so great that only the excess amount of the vitamins is secreted with larval food. It is also quite possible that these young nurse bees, while ingesting large amounts of pollen, may contaminate the larval food with the pollen grains while feeding the larvae. That an admixture of pollen with the food of larvae is accidental and not essential for the production of workers is apparent from the fact that adult bees can produce workers when supplied only with sugar solution, utilizing the stores of their own bodies for the synthesis of the larval food (Haydak, 1935).

To what an extent this drop in the vitamin content of the food of older worker larvae contributes to the underdevelopment of the older worker larvae is difficult to say without a further investigation of the requirements of the bee larvae for these vitamins. However, since the B group vitamins are necessary for growth, then their deficiency will bring the retardation in the development of a growing organism.

The results of these investigations show that there is not much variation in the vitamin content of the food of the queen larvae supplied during their growth. Recent observations by Taranov and Ivanova (1946) and of Taranov (1947) give a clue to the explanation of this phenomenon. In a colony preparing to swarm there is an excess of unemployed nurse bees. The swarm cells are started because of the discrepancy between the egg laying activity of the queen and the amount of the larval food produced. The latter, especially in the strong colonies, cannot be utilized as food either for the brood or the queen, and consequently the bees force the queen to deposit eggs in the queen cups. As soon as the queen larvae hatch, the unemployed nurse bees can lavishly supply the larvae with the food. In such a case, of course, a greater uniformity in the composition of the food could be expected, which actually exists as our investigations demonstrated.

Bioassays, using vitamin K deficient chicks, showed that royal jelly did not manifest any antihemorrhagic activity when fed for four days at the rate of one gram daily in water suspension with a supplement of ethyl laurate. The fatty acid ester was supplied in order to assure the presence of a fatty carrier in the intestines of the chicks.

Samples of beebread (pollen stored in the cells of combs by the bees) from 1938, 1939 and 1940 collections were also investigated for their antihemorrhagic activity. The prothrombin time was 2 min. 48 sec., 2 min. 51 sec., and 3 min. 2 sec., respectively, when 1 gm. of each beebread sample was supplied daily for four days during the curative period to vitamin K depleted chicks. This corresponds quite closely with the average prothrombin time of 2 min. 57 sec. for positive controls which received one microgram of synthetic vitamin K (2-methyl-1, 4-naphtoquinone in ethyl laurate) daily during the same period. The prothrombin time of the negative controls was 25 minutes.

It is of interest to note that mixed pollens brought to the hive by bees possess no vitamin K activity (Vivino and Palmer, 1944). Probably the bacteria which are instrumental in fermentation of beebread (pollen deposited in the cells of combs) synthesize vitamin K.

SUMMARY

The daily changes in the concentration of thiamine, riboflavin, niacin and pantothenic acid in royal jelly have been investigated. The thiamine content remained fairly constant, although values obtained by several investigators varied considerably. The riboflavin, niacin and pantothenic acid contents diminished as the age of queen larvae advanced, the sharpest drop being between the first and second day of larval life. The pantothenic acid content in the dry matter of royal jelly increased up to the fourth day of larval life, then it dropped considerably.

The thiamine content of the fresh food of worker larvae remained practically unchanged during their development. However, on the dry matter basis, there was a drop of 30% in the content of this vitamin in the food of older larvae. The concentrations of riboflavin, niacin and pantothenic acid in the food of 3- to 5-day old worker larvae were significantly diminished as compared with the food of 1- to 2-day old larvae.

It is suggested that the differences in the vitamin concentration of the food of older worker larvae as compared with that of the younger larvae are due to the fact that the incompletely developed pharyngeal glands of the young nurse bees feeding the older larvae do not produce the larval food of the same vitamin content as that secreted by the older nurse bees feeding the younger larvae.

Royal jelly did not exhibit any antihemorrhagic activity. One gram of beebread showed a vitamin K potency equivalent to one microgram of 2-methyl-1, 4 naphtoquinone.

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A NEW NAUCORID GENUS AND SPECIES FROM NEVADA

(Hemiptera)[1]

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Family **Naucoridae**

Subfamily **Limnocorinae**

Usingerinini tribus novus

Usingerina genus novum

Generic characteristics.—*Head* narrowly and briefly foveate on either side near anterior margins; antennae very short, not reaching inner anterior angles of eyes. *Pronotum* very short and broad, four times broader than long, posterolateral angles prominent, expanded and narrowly rounded. *Scutellum* with lateral margins elevated, sinuate at middle, the disc feebly depressed. *Hemelytra* possessing the most obviously distinct characteristics of the genus: claval suture wanting; claval and corial veins thickened and elevated; embolium prolonged into a single, sharp, long, curved spine at its posterolateral angle; membrane thickened, greatly reduced, merely a thin inverted "V," terminal width distinctly less than 50% of length. *Hindwings* greatly reduced, usually extending just slightly caudad of middle of second visible abdominal segment. *Abdominal segmentation* obsolescent in mid-dorsum.

Genotype.—*Usingerina moapensis* sp. nov.

Usingerina moapensis species nova

General appearance.—Broadly oval in outline, with a marked tendency toward lateral expansion of pronotum, embolia and abdomen; darker in color posteriorly, the mottling fading to light yellow anteriorly; size small, 5.25–6.0 mm. long and 4.5–5.0 mm. wide (at widest part of abdomen, excluding embolia).

Head.—White to whitish-yellow, with a variable development of brown dotting and suffusion; the most clearly defined pattern is one consisting of several series of brown punctures surrounded by brownish suffusion, each series forming a bar. One such strong bar lies laterally paralleling each inner eye margin; a medianly interrupted bar, curving slightly caudad, occupies the posterior edge of head; two pairs of short, longitudinal bars occupy the center of the head, the innermost pair consisting of a strong series of punctures, the outermost more in the

[1]This study was aided in part by a research grant from the University of Nevada.

nature of brownish suffusions with a tendency to spread out anteriorly; random dotting occurs in area between posterior interrupted bar and caudal ends of median central bars; surface generally polished and shiny, with punctures as indicated above. When oriented so that dorsal plane of head is perpendicular to line-of-vision (*i. e.*, the greatest amount of dorsum exposed to view), front of head is seen to be markedly protuberant forward; head more protuberant before eyes than behind eyes (see ratios 4 and 5). Anterior margin of head bearing a thin, shallow, faint foveate line near each eye. Eyes reddish-black; outer and posterior margins curving imperceptibly into each other; outer and anterior margins subangulate at junction; viewed posteriorly, eyes markedly protuberant above general head surface. Entire head, including eyes, characteristically deeply set into anterior pronotal margin, anterolateral eye margin nearly forming a continuous, smooth contour with anterolateral pronotal angle. Mouthparts darkening at tip.

Head ratios are

1) total length to width (including eye), 32: 50.
2) anterior distance between eyes to posterior distance, 34: 30.
3) posterior distance between eyes to greatest length of head posterior to this line, 30: 4.
4) eye length to width, 26: 11.
5) posterior distance between eyes to greatest length of head anterior to a line drawn between anterior gape of eyes, 30: 6.

Pronotum.—Dull, slightly roughened and irregular, with some large and small punctures, and patterned with brown dots and suffusions. Groundcolor whitish with a variable development of dotted patterns, occasionally with a faint rosy suffusion mediocentrally. When best developed, pattern shows an irregular, semitriangular area outlining and coloring the low, broad callus of pronotal disc to each side of the median line. Lateral pronotal expansions may be largely white, or strongly dotted with large, isolated, brown dots; posterior border may be similarly bare or marked. There is usually a broken series of brown dots along the medioanterior pronotal border. Broad "V"-shaped parallel rugosities in anteromedian portion of disc, flanked by the two calli. Lateral edges minutely crenulate, lacking any marginal pilosity. Pronotum strongly expanded laterally, percent of expansion (expressed in terms of straightline distance between anterolateral and posterior angles, and the longest line perpendicular to this baseline between baseline and pronotal lateral edge) between 34 and 35 (whereas in *Limnocoris signoreti*, such expansion is only between 21 and 22%). Posterolateral corners nearly right-angulate with apices rounded, some distance laterally removed from the posterior angles (which latter are weak and hardly apparent as slight sinuosities in the posterior pronotal margin near lateral edges; in *L. signoreti*, the posterolaterals are obsolescent, not evident by virtue of the smoothly curving edge of the pronotum, and the posterior angles proper are very near pronotal edge). Propleura blackish along borders, except posteriorly, yellowish centrally. Prosternal carina prominent anteriorly, topped by two equisized tubercles, best seen in lateral view.

Pronotal ratios are

1) width between anterolateral angles to width between posterolateral angles, 56: 116.
2) median length to greatest width, 29: 116.
3) width between anterolateral angles to distance between these angles and posterior base line of pronotum, 56: 44.

Scutellum.—Broadly "V"-shaped, largely whitish-yellow with brownish suffusions posteriorly and laterally, and generally with two prominent blackish spots at anterior margin between centrum and lateral angles; surface slightly roughened. Lateral margins elevated, sinuate at middle, disc feebly depressed. In normal position, *i. e.*, approximately on a plane surface with remainder of body, ratio of three sides, anterior and two laterals, respectively, is 52 : 38 : 38.

Hemelytra.—In alcoholic material, transparent enough for brownish abdominal color to show through, the raised, coarse claval and corial veins appearing white by comparison; in dried specimens, entire surface slightly roughened, whitish-crustose, considerable blackish showing through except around and on embolia and along raised veins, which are uninterruptedly whitish. Claval suture wanting. (In *L. signoreti*, the hemelytral veins are not raised, and claval suture is present). Membrane generally lacking white crustosity posteriorly and on left side, better seen in alcoholic than dried material, and as a consequence distinctly blacker. Membrane greatly reduced in size, forming a thin, inverted "V" which is less than 50% (60 : 26) as wide as long (whereas in *L. signoreti*, the well-developed membrane is considerably more than 75% (90 : 80) as wide as long). Embolium quite remarkable, laterally expanded and terminating posterolaterally in a long, sharp, posteriorly curved spine. Hemelytra strongly exposing lateral connexival margins which are slightly darker in color; posterolateral connexival angles non-spinose, each segment fitting tightly to the other, with no overlap or discontinuity of outline. Hemelytra barely attaining abdominal tip.

Venter.—The prothoracic venter has been discussed above. Remainder of thoracic venter rich yellow in alcoholic material, generally distinctly, but only slightly, lighter than abdominal venter. In dried specimens, the fine, dense pilosity of abdomen is quite evident, extending to sternal area and leg bases, remainder of thoracic venter being largely bare. Median carinae three in number, the anterior two foveate, the posterior one a low, inconspicuous ridge. Mesosternal carina the largest, its deep suctorial fovea large, round, lying centrally between fore- and mid-legs, and bordered anteriorly by a small, raised tubercle; metasternal carina smaller, lower, its suctorial fovea shallow and lying just posteriad to insertion of mesolegs; carina of first two visible abdominal segments thin, plate-like, anteriorly widened slightly posteriorly and extending beneath third ventral segment; carinal foveae blackish in contrast to yellow sternal plates.

Legs.—Prolegs with typical globular coxae, incrassate femora, thin, lancetlike tibia-tarsi which rest, when closed, in grooves along femoral surfaces; tibia-tarsi darkening at tip, in contrast to whitish femora and, when closed, not quite attaining proximad end of femora. Meso- and meta-legs similarly whitish with contrasting brown spines and spinules

arranged symmetrically along edges of the box-like femora and tibia (spines on tibia, spinules on femora). Meta-tibia and -tarsi thickly furred on inner margins with long, whitish hairs which form a solid swimming web. Meso- and meta-tarsi two-segmented, tipped with two large, brownish claws.

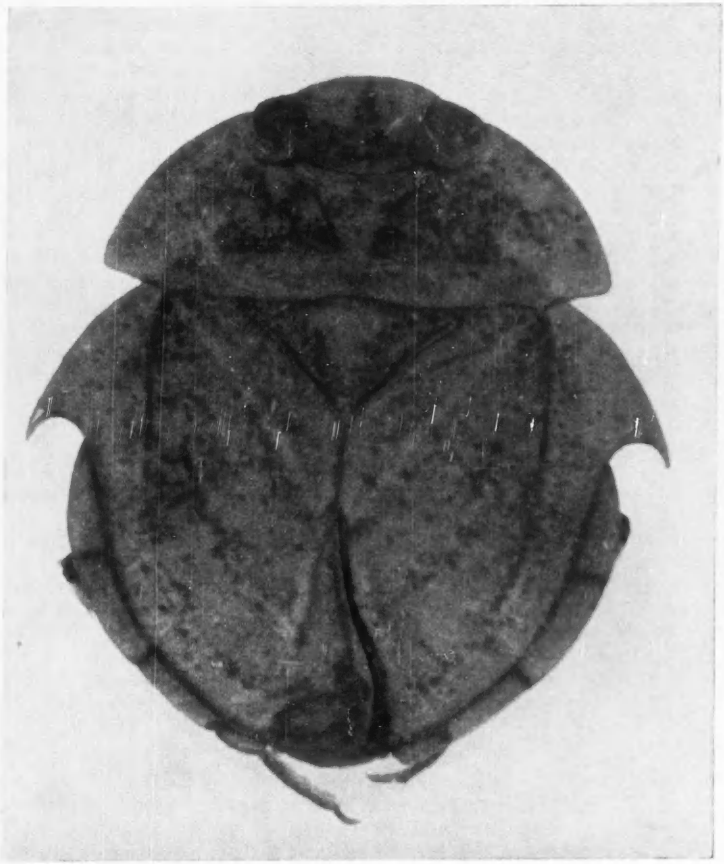

FIG. 1. *Usingerina moapensis* La Rivers, female allotype.

Type locality data.—NEVADA, Clark County (*Warm Springs* (outlet streams of Big Pool) 26–27 (xii) 48, el. 1700 ft.–LaR). Known only from the type locality.

Disposition of types.—Holotype, allotype and four paratypes in the California Academy of Sciences (San Francisco); paratypes in the

collections of Dr. Robert L. Usinger (Berkeley, California); Snow Museum, University of Kansas (Lawrence); U. S. National Museum (Washington); American Museum of Natural History (New York City); British Museum (London); Paris Museum (France); and the writer (Reno, Nevada).

Ecologic data.—Since I have already characterized the general aspects of the type locality in a previous paper (reference 5), the following notes will merely be supplementary. ***Usingerina moapensis*** was found only in swift warm water, varying in temperature from 89° F. to 75° F. (Stations A and B, respectively), the former representing a collecting locality but a few yards downstream from the source pool, the latter a locality some 100 feet farther downstream and a few yards up a small, cooler tributary which came from the Pelocoris marsh (see below). Hydrogen-ion concentrations as determined with a Hellige pocket comparator, gave a pH of 7.3 at Station A, and 7.5 at Station B. At the latter, *U.* ***moapensis*** was found with the omnipresent ***Ambrysus mormon*** Montandon 1909, the latter dominant, whereas at Station A, where *Usingerina* predominated, only an occasional *A.* ***mormon*** was present in the ratio of approximately 1 : 20. In this last situation, *A.* ***mormon*** was found most commonly in streaming vegetation along the shores and sides, while the gravel bed of the stream was occupied by large numbers of *U.* ***moapensis***. Although ***Pelocoris shoshone*** La Rivers 1948 was found a few yards away in a marsh drained by the small stream on which Station B was located, it did not seem to occur beyond the marsh, and there were no indications that *Usingerina* and *Pelocoris* occupied the same environment. This is not strange when the habitat preferences of the two are considered. The former is an habitue of gravel-bottomed swift streams, while the latter prefers the overhanging turf banks and shores of slow and still water. That it was not a matter of temperature is shown by the fact that *P.* ***shoshone*** was found in water of 83° F. (pH 7.3), well within the range of *U.* ***moapensis***. *Ambrysus* was also absent from the Pelocoris marsh, although *A.* ***mormon*** was in dominance in the small drain ditch mentioned above at the foot of the marsh, where the tiny stream fell turbulently down a steep 15-foot slope from the marsh to a small meadow, through which latter it meandered for a few yards before entering the main stream in which Station A was located.

In its bottom preferences, *U.* ***moapensis*** greatly resembles another markedly endemic naucorid, ***Ambrysus funebris*** La Rivers 1948 from Death Valley. Both species avoid stream bottoms where the water is slow enough to allow fine sand to accumulate, or swift enough to sweep gravel away; only in gravel areas of intermediate stream flow is either found, and then in large numbers, several commonly taken in one sweep of the seine.

The only other prominent associated invertebrates found in this small drainage complex at the time of winter collecting were a new species each of the dryopids *Stenelmis* and *Microcylloepus*, the former being found in both Pelocoris marsh and the swift, major streams occupied by *Usingerina*. The small cyprinodont fish *Crenichthys beileyi* (Gilbert) 1893 was the dominant vertebrate common to the

entire system, while the newly described cyprinid *Moapa coriacea* Hubbs & Miller 1948 was absent only from the marsh.

At the present time, it is difficult to place *Usingerina* phylogenetically. It is a characteristic limnocorine by virtue of its ventral foveate carinae, but differs so remarkably from any known *Limnocoris* (the only genus hitherto known in the subfamily) in other features that it warrants tribal separation.

Dr. Robert L. Usinger, to whom I dedicate the genus in appreciation of many past favors and in regard for his outstanding work in the family, was kind enough to compare the animal with type material of *Limnocoris* in the Montandon collection (British Museum), and pointed out (*in litt.*) that while its nearest geographical relative was *Limnocoris signoreti*, *U. moapensis* was structurally unlike any other members of the subfamily of his acquaintance.

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This is an old journal, formerly *The Wasmann Collector*, appearing under a new name and plan of publication. It will now be issued three times a year, with Spring, Summer, and Fall issues. The subscription rate is $5.00 a year. The present issue, the first under the new plan, is entomological to a large extent, with articles by D. Elmo Hardy, William Hovanitz, Edward L. Kessel, J. W. Tilden, Hermann Schmitz, George C. Steyskal, and Ira La Rivers. The four other articles in this issue deal with subjects in herpetology, invertebrate zoology, and botany. According to the editorial notice, contributions may deal with any field of academic biology but must be based on original investigations and published exclusively in this journal. Authors associated with the University of San Francisco are given priority in publication. The present managing editor is Edward L. Kessel.

—M. T. J.

HYDRELLIA (EPHYDRIDAE) AND SOME OTHER ACALYPTRATE DIPTERA REARED FROM POTAMOGETON[1]

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In an earlier paper (Berg, 1949) 32 species of insects intimately associated with plants of the genus *Potamogeton* in Michigan were listed. Species of plants and areas in which they were collected were recorded. The insects, including Diptera of 17 species, were dismissed with a few general remarks concerning aquatic adaptations, modes of hibernation, and injuries inflicted upon host plants. Biology and morphology of immature stages of nine species of Chironomidae reared from *Potamogeton* are discussed in another paper (Berg, 1950). Similar data regarding eight acalyptrate species will be presented here. Parasitic Hymenoptera reared from puparia of *Hydrellia* in this investigation represent nine species, seven of which appear to be new. Publication of data on the biology of these interesting insects will be deferred until they are described and specific names become available.

Larvae of the six species of *Hydrellia* included here seem to be directly related to *Potamogeton* species in three important respects. As leaf- and stem-miners they depend on these plants for food. The plant epidermis which conceals and shelters them apparently affords a considerable degree of protection. Circumstantial evidence indicates that *Hydrellia* larvae normally obtain oxygen from the intercellular gas spaces of these plants. No evidence of nutritive or protective relationships between *Potamogeton* and larvae of the two other acalyptrate flies was observed. It is apparent, however, that *Notiphila loewi* larvae regularly depend on these plants for sufficient oxygen to maintain life, and that specimens of *Hydromyza confluens* less frequently do the same.

I gratefully acknowledge the guidance and substantial assistance of Professor Paul S. Welch, identifications of four species and original descriptions of three others furnished by the late E. T. Cresson, Jr., formerly of The Academy of Natural Sciences of Philadelphia, and identification of *Hydromyza confluens* supplied by F. M. Snyder, of Orlando, Florida.

Hydrellia (Ephydridae)

It appears that the genus *Hydrellia* has been reported from all zoogeographic regions except the Neotropical. Cresson (1932) published taxonomic notes on 32 species from such widely separated regions as Australia, Formosa, South Africa, Europe, and North America, and his subsequent descriptions include two species from Hawaii (Cresson,

[1]Contribution from the University of Michigan Biological Station and from the Department of Zoology, University of Michigan.

1936). Becker (1926) discussed 35 European species and presented a bibliography of papers on the biology and immature stages of some of them. Cresson (1944) gave distributional notes and keys to 35 Nearctic species, and the taxonomy presented by him is followed in this pader.

All *Hydrellia* larvae of known habits are phytophagous. Those of most species mine in leaves and stems of aquatic plants, but others attack terrestrial plants, especially the grasses. Some larvae, such as those of *H. griseola* Fallen (De Ong, 1922, as *H. scapularis* Lowe; Wilke, 1924; Kortung, 1931) and *H. nasturtii* Collin (Marchall, 1903, as *H. ranunculi* Haliday; Collin, 1928; Taylor, 1928; Vayssiére, 1933) mine in the leaves of grains and water cress and sometimes assume economic importance. Hendel (1926), Hering (1935–'37), and Hennig (1943) reviewed the knowledge concerning immature stages of European *Hydrellia*.

The larvae of five European species of *Hydrellia* are known to live on *Potamogeton*. Thienemann (1916) reported *Hydrellia chrysostoma* Meigen from *P. lucens* and *H. nigripes* Meigen from *P. crispus*. Muller (1922) found *H. nigripes* mining in both *P. lucens* and *P. perfoliatus*. Brocher (1910) reared *H. modesta* Loew from an unspecified species of *Potamogeton*, and Thienemann (1916) found what was possibly the same species mining in the leaves of *P. natans*. Hering (1930) described *H. potamogeti* from material "aus Minen an Potamogeton erzogen," and (1935–'37) also reported *H. chrysostoma*, *H. nigripes*, and *H. fascitibia* v. Roser from *Potamogeton*. Wesenberg-Lund (1943) reported *H. modesta* from *P. natans*.

Very little has been published concerning immature stages of North American *Hydrellia*. Malloch (1915) figured the puparium and posterior part of the larva of *H. griseola* var. *scapularis* Lowe, and reported that the larva mines in *Panicum*. From a single specimen, Johannsen (1935) described the puparium of *H. griseola* var. *hypoleuca* Loew, but was unable either to distinguish it from that of variety *scapularis* or to name the food plant. Johannsen also described briefly a puparium believed to be that of *H. cruralis* Coquillet, and illustrated parts of the larva of *Hydrellia* sp. None of the scattered references to the occurrence of Nearctic *Hydrellia* larvae in *Potamogeton* (Moore, 1915; Frost, 1924; Cresson, 1934, 1944; Johannsen, 1935) specifically identifies both the fly and its food plant.

In the present investigation more than 800 specimens of *Hydrellia* were reared from larvae and pupae found in *Potamogeton*. These flies represent six species, of which *H. cruralis* Coquillet, *H. pulla* Cresson, and *H. caliginosa* Cresson were known before this study was undertaken, and *H. bergi* Cresson, *H. ascita* Cresson, and *H. luctuosa* Cresson have been described since from specimens reared in the pursuit of this research.

Eggs which were observed, those of *H. bergi*, *H. cruralis*, and *H. pulla*, are elongate, cylindrical, measuring approximately 0.5x0.15 mm. All have irregular longitudinal furrows in the chorion, and each possesses a bell-shaped projection bearing the micropyle near one end.

Mature *Hydrellia* larvae examined in this study, those of *H. ascita*, *H. bergi*, *H. cruralis*, *H. luctuosa*, and *H. pulla*, are similar in some respects. All are approximately cylindrical and tapered toward both

ends. In the third (last larval) instar, they range from 2.5x0.5 mm. to 6.0x1.0 mm. All are metapneustic, the only spiracles being slit-like openings near the tips of two sharp, hollow spines at the posterior ends of their bodies. Larvae are usually found with these spines inserted into plant tissue. Presumably they obtain oxygen from the intercellular gas spaces of plants by this means. The sclerotized anal plate, bearing a median longitudinal anal slit, is on the ventral surface a short distance anterior to these spines. On the ventral surface, anterior to the anal plate, there are eight well-defined and conspicuous spinulose areas used in locomotion (the "creeping welts" of authors). Anterior to them, three less prominent spinulose areas become progressively smaller and more diffuse toward the anterior end. The pharyngeal skeletons are relatively simple, and each bears a single mouth hook. This condition may not be constant for the genus, since Williams (1938) refers to the "pair of mouth hooks" of *H. williamsi* Cresson, and Wesenberg-Lund (1943) mentions the "Paar kleinen Mundhaken" of *H. modesta*. Anterior spiracles apparently are lacking. A pair of three-jointed sensory projections (the "antennae" of various authors) are borne near the anterior end of the body. Below these, just anterior to the mouth hooks, there is a pair of single-jointed tubercles which may also have a sensory function. All larvae possess some spinules or setae in addition to those on the creeping welts, but their sizes, numbers, and distribution patterns differ in larvae of different species.

Puparia of all species reared are light brown, subcylindrical, shorter and thicker than the larvae. They are 2.4–4.5 mm. in length and 0.7–1.5 mm. in maximum breadth. Vestiges of the larval respiratory spines, anal plates, creeping welts, and setal patterns are evident, and each puparium contains a larval pharyngeal skeleton and mouth hook. Puparia of *H. ascita*, *H. bergi*, and *H. caliginosa* differ from the other three species in being narrower and more elongate, tapering slowly and gradually toward the posterior end, and having the respiratory spines terminal. Puparia of *H. cruralis*, *H. pulla*, and *H. luctuosa* are more compact and taper more abruptly to their posterior ends. Their respiratory spines are subterminal, and their anal plates are more or less concave posteriorly. Emerging *Hydrellia* open their puparia by pushing off an anterodorsal portion ("dorsocephalic cap" of authors) including the terga of approximately the three thoracic segments. Unlike many emerging muscoid flies, which detach or at least loosen a corresponding ventral portion of their puparia containing the larval mouth hooks and pharyngeal skeletons, *Hydrellia* leave their puparia with ventral surfaces intact.

Newly emerged flies of these six species are hydrofuge, and they experience little difficulty in getting up through the surface film. They crawl up on some floating or emergent object and rest there for about five minutes, meanwhile brushing their bodies, especially the wrinkled wings, with their feet. Then the wings expand rapidly, reaching full size in about 45 seconds. They remain soft and useless for a few minutes after expanding. Adult flies alight and walk on the surface film without getting wet. They mate while resting on floating or emergent objects in the natural environment and lay their eggs on exposed portions of the food plant.

All *Hydrellia* mines encountered in this study are linear, and of uniform pale green color. They contain no conspicuous frass nor distinguishing marks caused by mouth hooks of the larvae. Those in leaf blades are usually near the midrib and parallel with it. Puparia, which are formed within larval mines, have the respiratory spines inserted in midribs or other vascular structures.

Hydrellia in various stages of their life cycles were brought into the laboratory for closer observation. Leaf-mining larvae thrived and pupated in finger bowls if fresh leaves were supplied as needed. Each puparium was cut out as soon as it filled with gas around the pupa and became buoyant, and was placed in about a centimeter of water in a small vial for individual rearing. Since the decay of excised plant material might produce conditions which would kill the pupa, I tried at first to take as little leaf tissue as possible with each puparium. It was found, however, that too little plant tissue may constitute a greater hazard than too much. The margins of the excised plant material become waterlogged. If it is so small that water penetrates to the respiratory spines, the puparium fills with water and sinks, and the pupa dies.

A higher percentage of adult flies can be obtained by keeping whole leaves or whole plants containing the puparia alive in screen-covered bowls or aquaria. If individual rearing is desirable and time for daily examination of the puparia is available, each puparium can be cut out when the pupa within it turns dark and transferred to a stoppered vial containing a small amount of water.

KEY TO LARVAE AND PUPARIA OF HYDRELLIA WHICH INFEST POTAMOGETON IN MICHIGAN

A. Larva relatively broad, maximum breadth about 16 per cent of length, shape similar to that of *H. cruralis* (Pl. I, fig. 8); puparium (Pl. III, figs. 1, 4, 5) fairly compact, with respiratory spines subterminal; dorsal setae at least in intersegmental grooves of all abdominal segments, apparent with low power of compound microscope; pharyngeal skeleton (Pl. II, figs. 1, 4, 5) with relatively long, narrow shank, dimension between its ventral projections at least 2.3 times the breadth midway between projections . . . . . B

B. Small species, mature larvae less than 4.8 mm. long; puparium (Pl. III, fig. 1) 3.5 x 1.1 mm. or smaller, tapered more gradually posteriorly than anteriorly, posterior margin of anal plate only slightly concave; dorsal setae abundant, generally dispersed, in irregular transverse rows; pharyngeal skeleton (Pl. II, fig. 1) pigmented throughout, with dorsal rods opaque black . . . . . **luctuosa**

BB. Larger species, mature larva more than 4.8 mm. long; puparium (Pl. III, figs. 4, 5) 3.5 x 1.0 mm. or larger, ovoid, abruptly tapered at both ends, posterior margin of anal plate strongly concave; dorsal setae fewer, concentrated in or near intersegmental grooves; pharyngeal skeleton (Pl. II, figs. 4, 5) less extensively pigmented, with dorsal rods colorless C

C. Mature larva (Pl. I, fig. 8) leaf-green; puparium (Pl. III, fig. 4) constricted between segments laterally, giving it a scalloped appearance; dorsal abdominal setae confined to narrow bands in intersegmental grooves; pharyngeal skeleton (Pl. II, fig. 4) usually colored as shown, rarely almost as light as that of *H. pulla* . . . . . **cruralis**

CC. Mature larva grayish cream color; puparium (Pl. III, fig. 5) without scalloped appearance, little if any constriction between segments; bands of dorsal abdominal setae wider, composed of distinct transverse rows; pharyngeal skeleton (Pl. II, fig. 5) always very sparsely pigmented . . . . . **pulla**

AA. Larva relatively narrow, maximum breadth about 9 per cent of length, shape similar to that of *H. bergi* (Pl. I, fig. 1); puparium (Pl. III, figs. 2, 3) elongate, tapered gradually to posterior end, with respiratory spines terminal; most abdominal segments usually without dorsal setae; pharyngeal skeleton (Pl. II, figs. 2, 3, 6) with relatively short, stout shank, dimension between its ventral projections less than 2.3 times the breadth midway between projections.....D

D. Larger species, puparium (Pl. III, fig. 3) usually more than 3.7 mm. long; pharyngeal skeleton (Pl. II, fig. 3) with distinct hump above cheliform spot, opaque black pigment continuous from cheliform spot posteriorly to dorsoventral fork, ventral rods longer than portion anterior to dorsoventral fork; mouth hook stout. Miner in stems and petioles but not in leaf blades...**bergi**

DD. Smaller species, puparium (Pl. III, fig. 2) usually less than 3.7 mm. long; pharyngeal skeleton (Pl. II, figs. 2, 6) without hump above cheliform spot, usually lacking opaque black pigment behind cheliform spot, ventral rods no longer (usually considerably shorter) than portion anterior to dorsoventral fork; mouth hook narrower. Miners in leaf blades only.....E

E. Dorsal setae lacking on all abdominal segments except first, second and last; pharyngeal skeleton (Pl. II, fig. 2) with only diffuse pigment behind cheliform spot.....**ascita**

EE. Dorsal setae at least in intersegmental grooves of all abdominal segments; pharyngeal skeleton (Pl. II, fig. 6) with opaque black pigment continuous from cheliform spot posteriorly to dorsoventral fork. Rare on *Potamogeton* in Michigan. Larva not observed.....**caliginosa**

Hydrellia cruralis Coquillet

The literature contains five records of *Hydrellia* larvae mining in *Potamogeton* which probably refer to *H. cruralis*. Moore (1915), working near Ithaca, New York, found the leaves of *P. amplifolius* mined by *Hydrellia* larvae. Frost (1924) cited Moore's work and stated, "*Hydrellia* n. sp. . . . mines in the leaves of *P. amplifolius*." Johannsen (1935) identified a single specimen presumably reared by Moore as *Hydrellia cruralis*. Johannsen suggested, however, that this specimen might have been incorrectly labelled, and this record remains in doubt. Cresson (1934) reported specimens of this species reared from *Potamogeton* sp. by Miss Gertrude Auxier, at Marlington, West Virginia. Cresson (1944) wrote of *H. cruralis*, "A relatively common northern species and which has been bred from pondweed in Michigan." While not so stated, it seems probable that this last record is based on material reared in this investigation and sent to Cresson for identification on September 1, 1940, March 18, 1941, and October 27, 1941.

During this study, *H. cruralis* was encountered more frequently than any other species of *Hydrellia*. Immature specimens were collected in Cheboygan, Emmet, Presque Isle, and Washtenaw Counties, Michigan. Larvae and pupae were found in the leaves of *Potamogeton alpinus*, *P. amplifolius*, *P. epihydrus*, *P. foliosus*, *P. gramineus*, *P. illinoensis*, *P. natans*, *P. nodosus*, *P. praelongus*, *P. Richardsonii*, and *P. zosteriformis*. In order of decreasing extent of infestation, the species which harbored the greatest numbers of *H. cruralis* were *P. amplifolius*, *P. Richardsonii*, *P. praelongus*, *P. gramineus*, *P. illinoensis*, and *P. nodosus*.

Egg (Pl. I, fig. 5).—Elongate, cylindrical, rounded at both ends; 0.5–0.6x0.12–0.17 mm.; central yolk mass opaque, cream color or faintly yellow; peripheral translucent zone wider at both ends; chorion delicately sculptured with irregular longitudinal furrows; micropyle terminal with lips flared, bell-shaped.

First Larval Instar (living).—Cylindrical and tapering abruptly at both ends; length 0.8–1.5 mm.; color uniform throughout; at first cream color, later becoming greenish; creeping welts relatively large, conspicuous; pharyngeal skeleton 0.15–0.19 mm. long, lightly pigmented throughout, darkly pigmented in same areas as those of third instar pharyngeal skeleton.

Second Larval Instar (living).—Shape unchanged except creeping welts relatively less conspicuous; length 1.5–3.0 mm.; color, leaf green; pharyngeal skeleton 0.27–0.29 mm. long, color pattern same as that of third instar pharyngeal skeleton.

Third Larval Instar (living) (Pl. I, fig. 8).—Length 3.0–5.5 or 6.0 mm.; breadth 0.7–1.0 mm.; color usually green, but variable, tends to match color of leaf; ventral setal pattern similar to that of puparium (Pl. III, fig. 4); dorsal setae in transverse rows confined to intersegmental furrows; setae dispersed laterally; each creeping welt fusiform, bearing 8 to 13 irregular transverse rows of spinules (Pl. I, fig. 6); anal plate ovoid; anal and postanal regions armed with spinules which become heavier and often double posteriorly; terminal respiratory spines set on small papillae; anterior end (Pl. I, fig. 7) supplied with paired, 3-segmented antennae, paired, one-segmented inferior tubercles, single median mouth hook, and post-oral tuft of fine hairs; pharyngeal skeleton (Pl. II, fig. 4) 0.40–0.62 mm. long, usually dark anteriorly as shown, but rarely almost as light as that of *H. pulla* (Pl. II, fig. 5), slight hump above cheliform black spot.

Puparium (Pl. III, fig. 4).—3.5–4.5x1.1–1.5 mm.; transparent golden brown; ovoid, subcylindrical, with laterally distinct intersegmental furrows giving puparium scalloped appearance; setal pattern as shown and as discussed for larva; anal plate crescent shaped with rounded ends; respiratory spines subterminal; pharyngeal skeleton as described for third instar larva; pupa within greenish, becoming olivaceous gray shortly before emergence.

Biology.—Eggs are deposited on or in the emergent portions of food plants. They are usually laid side by side in compact masses one layer deep, with all eggs of a mass oriented in the same direction. Apparently the female attempts to lay eggs in a concealed or protected situation. In breeding cages, most of the eggs were inserted into exposed intercellular air spaces in broken midribs of leaves and into torn openings in old mines. Others were found on strips of paper hanging over the water, on floating leaves, and, in a few instances, floating unattached. The oviposition which was observed was always at the surface of the water. In nature, egg masses were found in folds of leaves, in curled stipules, and on stems.

Within limits, speed of development increases with rising temperature. After one to five days of embryonation the completely formed larva is visible through the egg shell. The active pharyngeal skeleton is particularly apparent.

From two to seven days after the egg is laid, the larva cuts a slit in one end of the egg shell and emerges. It bores directly into the leaf and begins to mine in the mesophyll between the upper and lower epidermis. In two or three days the larva has grown to a length of 1.5 mm., and the first molt occurs. The exuviae, including the pharyngeal skeleton, are left in the mine.

Mines of *H. cruralis* in thin, submerged leaves of *Potamogeton* are equally visible from either side, and are most readily seen by examining the leaves with transmitted light. Mines in coriaceous floating leaves are just within the lower epidermis and are visible only from the lower side by use of reflected light.

The larva selects fresh food. If the leaf in which it is mining dies, it cuts through the epidermis, migrates to a greener leaf, and starts a new mine. Six second stage larvae which were mining in a leaf that had begun to turn brown entered a fresh, green leaf within 12 hours after it had been supplied.

That selection of newer, greener leaves also occurs in nature is evident from repeated observations on *Potamogeton Richardsonii*. A lower leaf often has a mine which contains the cast exoskeleton of the first stage larva. The mine in the leaf immediately above it contains exuviae of the second stage larva. The next leaf is mined but empty, and the fourth leaf contains the mature larva or the puparium.

Under laboratory conditions, the duration of the second stadium is about 5 to 8 days; that of the third, approximately 10 to 18 days. Then the mature larva usually takes a position where the mine is in contact with the midrib. The respiratory spines are inserted into the midrib, the body is contracted to assume the proportions of the puparium, and the body wall becomes sclerotized in this position. Two days later, the greenish pupa is visible within the transparent brown puparium. Ten to 14 days usually elapse between cessation of larval movement and emergence of the adult. During unusually hot weather, a group of adults once emerged only eight days after cessation of larval movement.

Emergence and early activities of the adult, observed during daylight hours ranging from 10:00 a. m. to 5:00 p. m., are as described for all five species in the introductory section. Young flies, prodded to test their avoiding reactions 15 minutes after emergence, escaped only by walking and by darting an inch or two. Since they could be forced to fall, it is evident that they could not fly effectively. This would seem to indicate that flies emerging in rough water must suffer considerable hazard of drowning. Twenty-five minutes after emergence they flew readily and well.

The adult feeds on *Potamogeton* leaves, chewing small holes in them. Scotland (1934) and Williams (1938) reported similar habits in closely related species of Ephydridae. However, the fly is not exclusively phytophagous. They often dismember and devour the bodies of other flies which die in the rearing bottle.

The reared flies mated within 24 hours after emergence, and laid eggs within 24 hours after mating. The life cycle was completed several times in the laboratory. It had a duration of 32–53 days; average of 24 records, 41 days.

In nature, the first adults of the season seem to appear at about the

24th of May in Washtenaw County, Michigan, and can be found continuously until approximately the middle of October. Thus, at least three generations per season are possible. However, no distinct emergence heights were observed. It seems that all stages of the life cycle may be found on any date during late spring, summer, and early autumn. A single plant from a summer collection may harbor eggs, all three larval instars, pupae, and empty puparia.

Larvae of *H. cruralis* were collected throughout the winter in green leaves of *P. amplifolius*, the winter form of which was described by Moore (1915) as follows: "In *P. amplifolius* the tip ends of the branches function as propagative structures. . . . These structures appear in the autumn developing only at the tips of branches. The internodes are short and thick and densely packed with starch. At the end there are a few partially unfolded leaves which continue to grow slowly or, at least, remain green all winter. These rapidly expand when the roots develop in the spring and the entire structure forms an effective and rapid means of propagation."

When other leaves of the food plant turn brown and begin to disintegrate in autumn some larvae migrate to these propagative structures and enter the new leaves. They become less and less active as the water cools, finally reaching a state of quiescence. Specimens representing all three larval instars were found thus hibernating in plants beneath the ice, but no other stages of the life cycle were collected during the winter. Specimens collected as larvae in midwinter and placed in an aquarium at laboratory temperature soon became active, resumed feeding, pupated, emerged, mated, and laid fertile eggs. In one instance, the larvae which hatched from such winter eggs were also reared to maturity.

Muller (1922), failing to find the young larvae of *H. flavicornis* Fall., speculated on where and how young *Hydrellia* larvae live. Williams (1938) watched the development of a single laboratory-hatched specimen of *H. williamsi*, but to date very little is known concerning the biology of young *Hydrellia* larvae of any species.

Observations on laboratory-hatched and wild specimens of *H. cruralis* indicate that failure to find first and second stage larvae of *Hydrellia* in nature is probably due chiefly to the great difficulty involved in seeing them. They match the color and optical density of leaves so perfectly that it is very difficult to find them even by carefully examining each leaf with a dissecting microscope. After the larva has been located by detecting movements of its minute pharyngeal skeleton and mouth hook it usually takes a few more seconds of careful scrutiny to distinguish the outline of its body.

The problem is further complicated in fresh winter collections because the torpid larvae make no movements which might disclose their presence. Within 24 hours after winter collections have been brought into the laboratory some larvae can usually be found actively mining in the rolled lower regions of partially opened leaves. Careful examination usually results in finding only a small fraction of the larvae present, however, and it seems advisable to keep all winter collections in aquaria at room temperature for three weeks before making a final check for the presence of *Hydrellia*.

Some *P. amplifolius*, collected under ice 12 inches thick at Third Sister Lake, Washtenaw County, Michigan, January 9, 1941, was carefully examined after it had stood in a warm laboratory for 24 hours. Four actively mining larvae of *H. cruralis*, three third and one second instar, were found and put into a rearing bottle. The examined plant material was placed in an aquarium which was freshly made up with tap water and kept at laboratory temperature. On January 29, 20 days after the collection was made, a re-examination of the growing plants revealed 11 third stage larvae and 23 puparia of this species.

Hymenopterous parasites of five species were reared from puparia of *H. cruralis*. The braconids *Ademon niger* (Ashmead) and *Chorebidea* sp. 1, and the diapriid *Trichopria columbiana* (Ashmead) were encountered occasionally to commonly during their respective seasons. Single specimens representing *Chorebidella* sp. and an unidentified genus of the family Pteromalidae were also obtained.

Hydrellia pulla Cresson

Johannsen (1935) stated that Moore had reared a specimen of *Hydrellia pulla* from *Potamogeton* near Ithaca, New York. The only other published note concerning the biology of this species appears to be that of Cresson (1944), who wrote, "A scarce northern species, associated with pondweed." This record was probably based partly on Johannsen's published statement, and partly on material reared in this investigation and sent to Cresson for identification on September 1, 1940, March 18, 1941, and October 27, 1941.

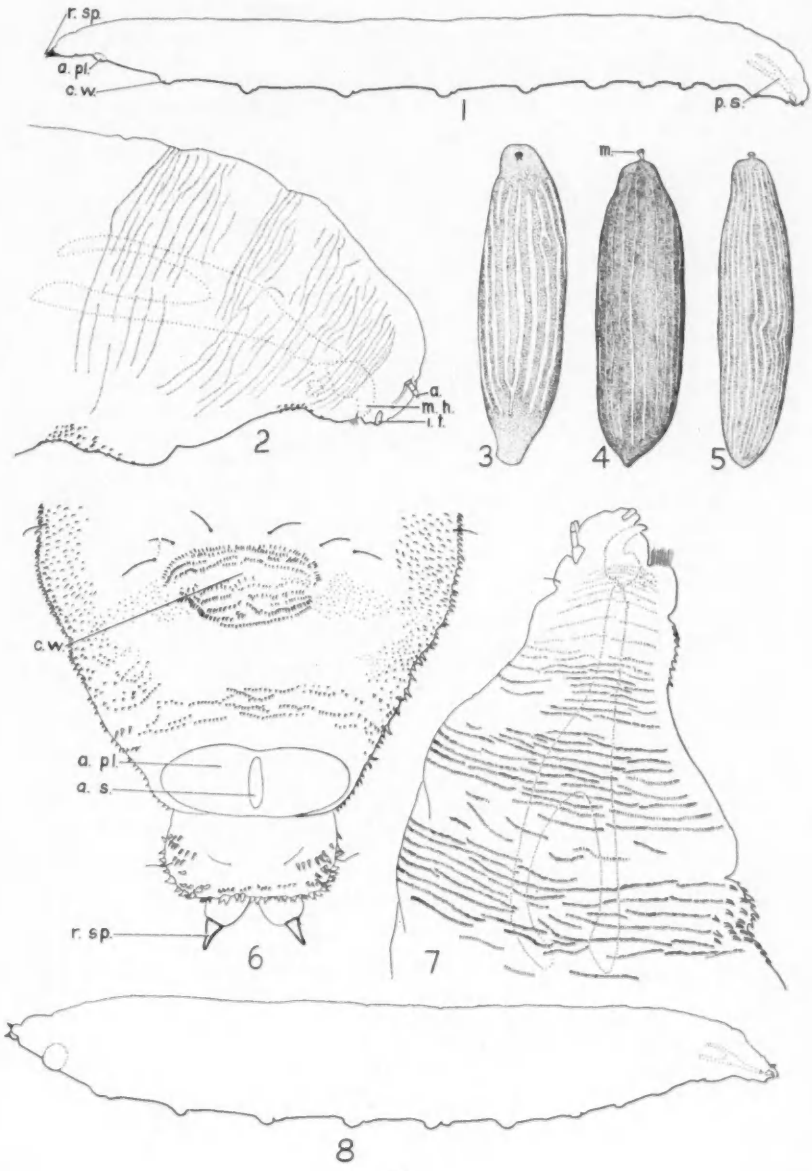
Specimens of this species were collected in Cheboygan and Washtenaw Counties, Michigan. Larvae mine in the leaves of *Potamogeton amplifolius*, *P. gramineus*, and *P. Richardsonii*.

Egg (Pl. I, fig. 4).—General shape like that of *H. cruralis* except for greater thickness, and end opposite micropyle is pointed; 0.57–0.65x 0.18–0.22 mm.; central yolk mass opaque, grayish brown color of variable intensity; surrounded by narrow translucent zone; longitudinal sculpturing of chorion more vague and obscure than on *H. cruralis* eggs; terminal micropyle projection longer than that of *H. cruralis*.

First Larval Instar (living).—Cylindrical and tapering abruptly at ends, with creeping welts conspicuous, protruding; length 1.0–1.8 mm.; color grayish cream throughout; apparently 12-segmented; pharyngeal skeleton 0.18–0.22 mm. long, darkly pigmented in same areas as those of third instar pharyngeal skeleton, with diffuse pigment throughout all other regions.

EXPLANATION OF PLATE I

Eggs and larvae of *Hydrellia* (Ephydridae). FIG. 1. *Hydrellia bergi:* Lateral view of whole larva. FIG. 2. *H. bergi:* Lateral view of anterior end of larva. FIG. 3. *H. bergi:* Egg. FIG. 4. *Hydrellia pulla:* Egg. FIG. 5. *Hydrellia cruralis:* Egg. FIG. 6. *H. cruralis:* Ventral view of posterior end of larva. FIG. 7. *H. cruralis:* Lateral view of anterior end of larva. FIG. 8. *H. cruralis:* Lateral view of whole larva. a, antenna; a. pl., anal plate; a. s., anal slit; c. w., creeping welt; i. t., inferior tubercle; m., micropyle; m. h., mouth hook; p. s., pharyngeal skeleton; r. sp., respiratory spine.


r. sp.
a. pl.
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1
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m. h.
i. f.
2
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c. w.
a. pl.
a. s.
r. sp.
6
7
8

Second Larval Instar (living).—Color and shape unchanged except creeping welts less conspicuous; length 1.8–3.5 mm.; pharyngeal skeleton 0.28–0.35 mm. long, with color pattern same as that of third instar.

Third Larval Instar (living).—Color and shape unchanged, the latter like that of *H. cruralis* (Pl. I, fig. 8); length 3.5–6.6 mm.; breadth 0.8–1.1 mm.; ventral setal pattern similar to that of puparium (Pl. III, fig. 5); transverse rows of dorsal setae tend to concentrate toward intersegmental furrows, but more dispersed than on *H. cruralis*; setal rows curved and running in various directions laterally; posterior end similar to that of *H. cruralis*, (Pl. I, fig. 6) with terminal respiratory spines short, anal plate ovoid, but no heavy, double spinules on postanal region; anterior end shaped like that of *H. cruralis*, with three-segmented antennae, one-segmented inferior tubercles, single median mouth hook, and postoral tuft of fine hairs; pharyngeal skeleton and mouth hook (Pl. II, fig. 5) light colored, and lacking hump above cheliform black spot, pharyngeal sclerite 0.50–0.65 mm. long.

Puparium (Pl. III, fig. 5).—3.5–4.5x1.0–1.5 mm.; transparent light brown; ovoid, subcylindrical, lacking distinct intersegmental furrows and scalloped appearance of *H. cruralis*; setal pattern as shown and as discussed for larva; anal plate strongly concave posteriorly with bluntly rounded ends, and not so crescent shaped as on *H. cruralis*; respiratory spines subterminal; pharyngeal skeleton (Pl. II, fig. 5) as described for third instar; pupa light colored, appearing yellowish brown through brown puparium, then turning darker and becoming almost black shortly before emergence.

Biology.—Eggs were found in nature during June, July, and August on floating or emergent portions of food plants. They are usually laid, like those of *H. cruralis*, side by side in compact masses one layer deep, often in such concealed situations as folds of leaves, ends of old mines, and in sheathing stipules. In the laboratory, they were laid on emergent parts of the food plant, free on the water surface, and stuck to the side of the rearing bottle.

Embryonation is accelerated by increased temperature. From two to four days after oviposition, the active larva becomes visible through the egg membranes. The eggs hatch in four to six days.

The newly hatched larva enters a leaf and begins to mine at once. In four to eight days it has grown to a length of about 2.2 mm., and the first molt occurs. Six to 10 days later, the larva molts again. The exuviae, including pharyngeal skeletons, of the first two instars are left in the mine.

Remarkable concentrations of *Hydrellia* sometimes result from the larva's tendency to migrate into younger, greener leaves. Three young

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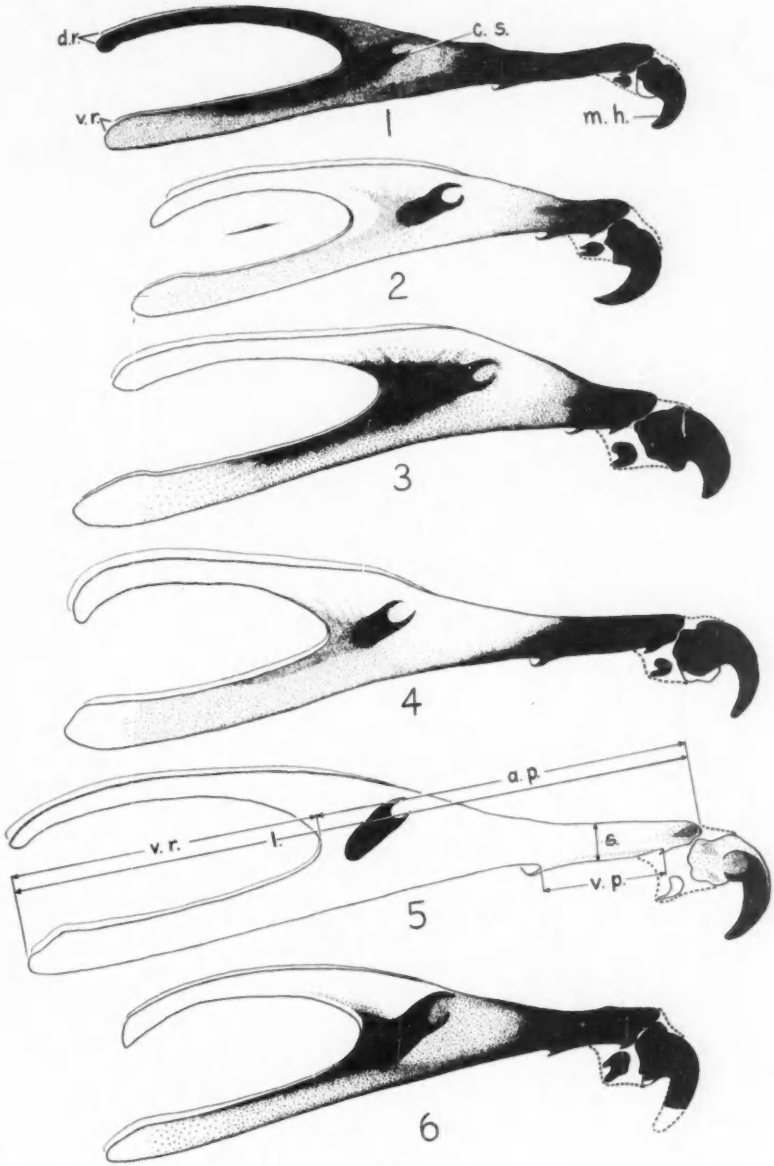
EXPLANATION OF PLATE II

Pharyngeal skeletons and mouth hooks of *Hydrellia* (Ephydridae). FIG. 1. *Hydrellia luctuosa*. c. s., cheliform spot; d. r., dorsal rods; m. h., mouth hook; v. r., ventral rods. FIG. 2. *Hydrellia ascita*. FIG. 3. *Hydrellia bergi*. Fig. 4. *Hydrellia cruralis*. FIG. 5. *Hydrellia pulla*. a. p., length of portion of pharyngeal skeleton anterior to dorso-ventral fork; l., total length of pharyngeal skeleton; s., breadth of shank; v. p., dimension between ventral projections; v. r., length of ventral rods. FIG. 6. *Hydrellia caliginosa*.


d.r.
c. s.
v.r.
m. h.
1
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a.p
v. r.
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s.
v. p.
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6

leaves of *P. amplifolius* collected May 3, 1942, at Third Sister Lake, Washtenaw County, Michigan, contained 8, 10, and 12 puparia, respectively. Each plant was composed of several tattered, brown leaves formed the previous autumn and one or two new green ones. Mines in the old leaves were empty, the larvae having migrated into the new leaves.

Under laboratory conditions, the duration of third stadium is approximately 8 to 15 days. Then the larva usually extends the mine into contact with the midrib and parallel with it, inserts the respiratory spines into the midrib, and contracts the body to assume the size and shape of the puparium. The body wall sclerotizes, and two days later the light colored pupa is visible within the puparium. Ten to 15 days usually elapse between cessation of larval movement and emergence of the adult.

It may be that emergence, which was observed repeatedly through a dissecting microscope, is hastened by radiations from the microscope lamp. A remarkably high percentage of emergences were witnessed when compared with the percentage of time that puparia were under observation. In this process there are intervals of activity and of rest. The imago grips the wall of the puparium with all six tarsi and pushes forward until an anterodorsal portion of the puparium ("dorsocephalic cap" of authors) is detached and the fly crawls out.

Newly emerged adults are unable to fly until a few minutes after their wings have expanded. Young, flightless adults of *H. pulla* avoid the heat and intense light of the microscope lamp by crawling onto the shaded sides of available objects. Adult flies in the rearing bottle exhibited the activities described in the general section and for *H. cruralis*.

Laboratory reared *H. pulla* mated and laid eggs within 48 hours after emergence. Life cycles observed had a duration of 34 to 55 days; average of seven records, 43 days.

Seasonal aspects of *H. pulla* are similar to those reported above for *H. cruralis*. Adults are found in Washtenaw County, Michigan, continuously from late May through early October. No emergence heights were observed. This species passes the winter as a torpid larva in the leaves of its food plants.

Apparently *H. pulla* is much less susceptible to parasitism than any other species of *Hydrellia* studied in this investigation. Although *pulla* was often observed closely associated with *H. cruralis*, the two braconid parasites *Chorebidea* and *Ademon*, so frequently reared from the latter, were never encountered in puparia of the former.[2] The only species of parasite reared from puparia of *H. pulla* was the diapriid *Trichopria columbiana* (Ashmead).[3]

[2]Seven puparia of *H. pulla* and five puparia of *H. cruralis* were found in a single leaf of *P. amplifolius* collected in Third Sister Lake, Washtenaw County, Michigan, May 3, 1942. There was no case of parasitism among the 80 *H. pulla* puparia in this collection, but 12 of the 71 *H. cruralis* puparia harbored braconid parasites.

[3]It is evident that *Hydrellia* may be used as food by fishes. The stomach of a bluegill (*Lepomis macrochira*) caught by R. C. Ball in Third Sister Lake, August 13, 1940, contained seven puparia of *H. pulla*. In another bluegill stomach, puparia of both *H. pulla* and *H. cruralis* were found.

Hydrellia luctuosa Cresson

Hydrellia luctuosa was described (Cresson, 1942) from specimens reared in this study. It appears that the only other published note on this species is that of Cresson (1944), who wrote, "A rare species from Michigan, reared from pondweed." It seems probable that this record was based solely upon the type series which was reared during the summer of 1941 and sent to Cresson during October of that year.

Specimens of *H. luctuosa* were collected in several localities in Cheboygan County, Michigan. In the order of preference, food plants of the larvae seem to be *Potamogeton alpinus*, *P. zosteriformis*, *P. Richardsonii*, *P. amplifolius*, and *P. natans*, infestation of *P. amplifolius* and *P. natans* being relatively uncommon.

Egg and first larval instar were not observed.

Second Larval Instar (living).—2.0–3.2 mm. long; relatively stout; grayish cream color; pharyngeal skeleton similar to that of third instar in shape and color, 0.26–0.31 mm. long.

Third Larval Instar (living).—Shape and color unchanged, similar to those of *H. pulla*; about 4.2x0.6 mm.; ventral setal pattern as shown for puparium (Pl. III, fig. 1); dorsal and lateral setae abundant, in irregular transverse rows, generally dispersed; pharyngeal skeleton (Pl. II, fig. 1) slender, heavily pigmented, 0.40–0.48 mm. long.

Puparium.—(Pl. III, fig. 1) Transparent brown: 2.7–3.6x0.7–1.1 mm.; taper toward posterior end more abrupt than that of *H. bergi* and *H. ascita*, not so abrupt as *H. cruralis* and *H. pulla*; respiratory spines subterminal; setal pattern as shown and described for larva; enclosed pharyngeal skeleton (Pl. II, fig. 1) as described for larva.

Biology.—The eggs were not found in nature, and attempts to secure them from reared flies were unsuccessful.

The larvae mine in the mesophyll layer between the upper and lower epidermis of thin submerged leaves. When, rarely, they mine in floating leaves they work just within the lower epidermis and are visible only from the under side.

Little is known about the seasonal behavior of *H. luctuosa*. Puparia appeared in nature as early as July 3 in 1941, and both larvae and puparia could be found throughout July and August.

H. luctuosa is parasitised by the diapriid *Trichopria columbiana*, and by the braconid *Dacnusa* sp. 2.

Hydrellia bergi Cresson

Hydrellia bergi was described (Cresson, 1941) from specimens reared in this investigation. The only other published reference to this species seems to be that of Cresson (1944), who wrote, "Not a common species, and known only from Michigan, where it has been bred from pondweed."

Specimens of *H. bergi* were collected in Cheboygan, Presque Isle, and Washtenaw Counties, Michigan. Larvae and pupae were extracted from longitudinal mines just within the epidermis of stems, quill-like submerged leaves, and petioles of *Potamogeton natans*, but were not found in leaf blades. A few larvae were also collected from mines in stems of *P. Richardsonii* and *P. zosteriformis*.

Egg (Pl. I, fig. 3).—Elongate, cylindrical, with ends more nearly truncate than those of *H. cruralis* and *H. pulla*; 0.6–0.7x0.17–0.20 mm.; longitudinal sculpturing of chorion very evident on exposed side of egg, but obscure on attached side; micropyle inconspicuous, subterminal, on exposed side.

First Larval Instar (known from exuviae only).—Pharyngeal skeleton and mouth hook shaped and colored like that of third instar (Pl. II, fig. 3); pharyngeal sclerite 0.16–0.18 mm. long.

Second Larval Instar (living).—Shape similar to third instar, relatively narrower than that of *H. cruralis*; length 1.5–3.0 mm.; color greenish yellow; pharyngeal skeleton similar to that of third instar except for small size; pharyngeal sclerite 0.25–0.27 mm. long.

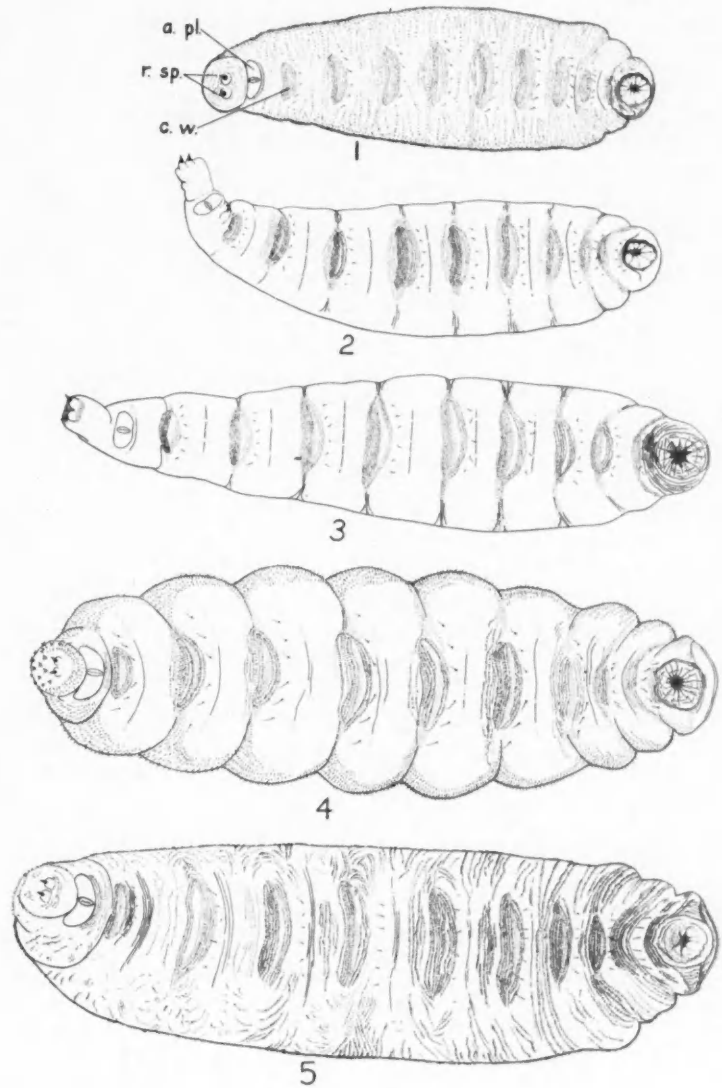
Third Larval Instar (living).—(Pl. I, fig. 1) Relatively narrower and more elongate than that of *H. cruralis*, *H. pulla*, and *H. luctuosa*; 5.2x0.58 mm.; color predominantly greenish yellow, with intestinal contents often showing through as a deep brown, surrounded by yellow orange area; ventral setal pattern similar to that of puparium (Pl. III, fig. 3); setae generally distributed over dorsal and lateral surfaces of pseudocephalic segment (Pl. I, fig. 2); posteriorly, dorsal and lateral setae occur only in bands in intersegmental furrows; the bands gradually become narrower posteriorly, the last one which completely girdles larva being in furrow between first and second abdominal segments; on following segments setae almost solely ventral, except on last, which is completely girdled by somewhat larger spinules; anterior end provided with structures illustrated and discussed under *H. cruralis*; pharyngeal skeleton and mouth hook (Pl. II, fig. 3) relatively stout, with definite hump above cheliform spot, the posterior boundary of which is obscured by concentration of black pigment; pharyngeal sclerite 0.42–0.52 mm. long; posterior end more gradually tapered than that of *H. cruralis*, with relatively longer respiratory spines; anal plate ovoid.

Puparium (Pl. III, fig. 3).—3.5–4.5 x 0.8–1.0 mm.; transparent light brown; distinctly more elongate and more gradually tapered posteriorly than that of *H. cruralis*, *H. pulla*, and *H. luctuosa*; respiratory spines terminal; anal plates ovoid with no concavity of posterior margin; setal pattern as shown and as described for larva; enclosed pharyngeal skeleton and mouth hook as figured and described for larva; pupa light colored at first, then developing pigment in compound eyes, then in body generally, becoming predominantly black before emergence.

Biology.—Eggs of *H. bergi* were deposited in the laboratory on stems of *P. natans* and attached to the side of the rearing bottle. In nature a similar egg mass was found attached to a stipule of *P. natans*. Unfortunately, none of these eggs hatched, and no information was obtained concerning embryonation, hatching, nor the newly hatched larva.

EXPLANATION OF PLATE III

Puparia of *Hydrellia* (Ephydridae). FIG. 1. *Hydrellia luctuosa*. FIG. 2. *Hydrellia ascita*. (Lateral curvature near posterior end of no significance.) FIG. 3. *Hydrellia bergi*. FIG. 4. *Hydrellia cruralis*. FIG. 5. *Hydrellia pulla*. Abbreviations as in Plate I.


a. pl.
r. sp.
c. w.
1
2
3
4
5

In biological aspects, larvae of *H. bergi* seem quite similar to those of *H. cruralis* and *H. pulla*. Since the stems in which they mine are thick and opaque, these larvae cannot be found by examination of plants with transmitted light. Some mines can be located because the epidermis covering them appears somewhat collapsed, and larvae were found only after opening such mines. Puparia can often be detected without dissection of plant tissue because the epidermis over them bulges outward and the dark color of mature pupae is often visible through the epidermis.

Just before pupation the larva provides for emergence of the adult by cutting a U-shaped incision in the epidermis of the stem. A similar provision is made by the larva of *Hydromyza confluens* Loew (Needham, 1908; Welch, 1914), a cordylurid species which pupates in petioles of the yellow water lily. The incisions made by larvae of these two species are similar in several respects. They are always cut in the epidermis covering the anterior ends of puparia. Both types are of such form as to produce lids which open readily when gentle pressure is applied from within. Both types of lids are attached along the side nearest the posterior end of the puparium.

Provisions for emergence in the two species differ in the following respects: (1) The incision made by *H. confluens* is in the shape of an arc extending approximately two-thirds the circumference of a circle (Welch, 1914) and has convergent ends; the incision made by *H. bergi* produces a U-shaped lid which is elongated in the longitudinal axis of the puparium and has parallel or slightly divergent ends. (2) The epidermal incision made by *H. bergi* is continuous, leaving no strands of tissue to hold the lid closed until the time of emergence. (3) Since *H. bergi* pupates just beneath the epidermis, there is no passage communicating between puparium and epidermal incision like that provided by *H. confluens*, which pupates deeper within the plant tissue.

H. bergi is the only species of *Hydrellia* herein discussed which provides for emergence of the adult in the manner described above. This is probably correlated with the fact that all other species of the genus reared during this investigation pupate in leaves, the epidermis of which is relatively thin and delicate and easily ruptured by the escaping imago.

In a few instances, puparia of this species were found with only their respiratory spines and posterior ends embedded in the *Potamogeton* stem, the greater part of these puparia protruding nakedly into the water. Such uncovered puparia were invariably found in leaf axils, where they looked very much like axillary buds. Perez (1901) described a similar habit in what appears to be a species of *Hydrellia* living on "*P. pectinatum*" in Europe.

In seasonal aspects, *H. bergi* is similar to the other species of *Hydrellia* discussed here. Emergence is not in well defined broods, but seems to be continuous throughout the summer. Both larvae and pupae were found in all eight collections of *P. natans* spread through two summer months in 1940 as follows: June 10, 20, 29, July 7, 20, 28, August 1, 9. Apparently this species passes the winter as a larva on the food plant. Larvae were taken in Washtenaw County, Michigan, on May 3, 1941, several weeks before adults were collected in nature.

Braconidae of three species, *Ademon niger* (Ashmead), *Chorebidea* sp. 2, and *Dacnusa* sp. 1, and the diapriid *Trichopria columbiana* (Ashmead) were reared from puparia of this fly.

Hydrellia ascita Cresson

Hydrellia ascita was described (Cresson, 1942) from flies which I reared during this study. Apparently the only other published reference to this species is that of Cresson (1944), who wrote, "Bred from pondweed of Michigan. Rare."

Specimens were found in several localities in Cheboygan County, and in Carp River, Emmet County, Michigan. Larvae mine in leaves of *Potamogeton alpinus*, *P. amplifolius*, *P. epihydrus*, *P. foliosus*, *P. illinoensis*, *P. Oakesianus*, *P. Richardsonii*, and *P. zosteriformis*.

Cresson designated only the flies reared from *P. alpinus* (—*P. tenuifolius*) collected at Nigger Creek, Cheboygan County, in his type series, and he identified the others as a variety of *H. ascita*. I am not able to distinguish larvae and puparia of the variety from those of the typical *H. ascita*.

During the summer of 1949, while Research Associate at Cranbrook Institute of Science, Bloomfield Hills, Michigan, I reared flies of this species extensively from *P. foliosus* growing in Sodon Lake, Oakland County.[4] Parasitic Hymenoptera representing a species not previously associated with *H. ascita* were reared at Sodon Lake, the first instar larva of *H. ascita* was observed, and additional information was gained concerning mining and pupation of the larvae within the very narrow leaves of *P. foliosus*.

Egg masses resembling those of other species of *Hydrellia* were found on leaves of *P. alpinus*. Since the source of these eggs is unknown, and since they failed to hatch, their identity remains in doubt.

First Larval Instar.—About 0.8–1.2 mm. long; anterior seven intersegmental furrows each with a band of setae; creeping welts relatively larger and more conspicuous than on older larvae; pharyngeal skeleton fuscous throughout except for black cheliform spot and mouth hook, 0.16–0.18 mm. long.

Second Larval Instar (living).—Similar to third instar in shape and color; length 1.2–2.4 mm.; pharyngeal skeleton colored like that of third instar, 0.23–0.29 mm. long.

Third Larval Instar (living).—Quite similar to *H. bergi* (Pl. I, fig. 1) in shape, color, and setal patterns, but smaller; 2.4–5.0x0.6–0.8 mm.; pharyngeal skeleton and mouth hook as shown (Pl. II, fig. 2), 0.35–0.49 mm. long.

Puparium (Pl. III, fig. 2).—Very similar to that of *H. bergi* in shape, color, and setal patterns, but smaller; 2.8–3.7 mm. long; enclosed pharyngeal skeleton and mouth hook as illustrated for third instar; pupa at first light, becoming dark gray before emergence.

Mines of *H. ascita* in thin submerged leaves are equally visible from

[4]Some doubt may remain concerning the identity of these specimens, which were obtained after Cresson's death. I find no consistent differences between these larvae and puparia and those of the collections which yielded the types of *H. ascita*. G. C. Steyskal, who has compared the adults with paratypic specimens of *H. ascita*, concurs in this identification.

either side and quite readily found by examination of the leaves with transmitted light. The larvae seem to prefer the mesophyll of such flaccid leaves. It is possible that they mine just within the lower epidermis of the coriaceous floating leaves of *P. Oakesianus* only because of failure to find their preferred food. *Oakesianus* has no flaccid leaves, and no other species of *Potamogeton* occur in the bogs in which it was collected.

The occurrence of a miner in leaves as narrow as those of *P. foliosus*, which average about 1.0 mm. wide in Sodon Lake, seems quite remarkable. The mine of a mature *H. ascita* larva usually occupies the entire width of the leaf. Since all mesophyll tissue is removed and only a sheath of thin epidermis remains, the mined leaf often shreds and disintegrates even before the puparium which was formed within it can produce an adult fly. Probably the puparia found floating unattached in the lake had been anchored only by insertion of respiratory spines into the leaf midrib, and were liberated by disintegration of the entire leaf.

If the pupae within are well matured before detachment of the puparia they may yield adult flies. However, most puparia float with respiratory spines below the surface film. I found in the laboratory that, unless emergence occurs in a day or two, detached puparia usually fill with water and sink.

Most *H. ascita* larvae which live in leaves of *P. foliosus* avoid the danger of detachment by a maneuver never observed and evidently unnecessary for larvae living in the other food plants listed. These mine down to the leaf base, turn about, and insert their respiratory spines into the stem before pupation. More than 95 per cent of the puparia observed were in this position. Puparia thus anchored usually remain attached even when the leaves which originally enclosed them disintegrate. In Sodon Lake, it was not uncommon to find three or four puparia projecting nakedly into the water from a stem of *P. foliosus* six or eight inches long.

Although their positions might suggest that these puparia were formed by stem-mining larvae which had projected their anterior ends from the mines before pupation, there is good evidence to the contrary. While larvae of all three instars were found mining in leaves, neither larvae nor larval mines were observed in stems. Puparia were invariably located at nodes. Some young ones were completely enclosed in leaf epidermis, and shreds of epidermis still adhered to many older puparia.

Like other *Hydrellia* discussed here, this species seems to have no clearly defined emergence heights. Larvae and puparia were collected in Cheboygan County during the last half of July and first half of August in 1946 and in Oakland County throughout both of these months in 1949.

H. ascita is parasitized by 2 species of Braconidae, *Chorebidella* sp. and *Dacnusa* sp. 3, and by the diapriid *Trichopria columbiana*.

Hydrellia caliginosa Cresson

Hydrellia caliginosa was described (Cresson 1936) from specimens collected on the yellow water-lily in Maine. The only published ref-

erence to the biology of this species appears to be that of Cresson (1944), who wrote, "A rare species from Maine and Michigan. It is associated with spatter dock and pondweed." While not so stated, it seems probable that the latter record is based upon a single specimen reared in this study and sent to Cresson for identification on October 27, 1941.

The puparium of the specimen referred to above was extracted from a mine in a leaf of *P. praelongus* taken at Ocqueoc Lake, Presque Isle County, Michigan, on July 13, 1941. Since this specimen is the only *H. caliginosa* among the hundreds of *Hydrellia* encountered during this study, it appears that the species either is rare in Michigan or that it normally infests some other plant.

No further information was obtained concerning the biology of this species. Neither egg nor larva was observed.

Puparium.—Transparent brown; 3.45x0.95 mm.; elongate and tapering gradually to the posterior end like those of *H. bergi* and *H. ascita*; respiratory spines terminal; dorsal setae at least in intersegmental grooves of all abdominal segments; ventral setal pattern similar to that of *H. ascita* (Pl. III, fig. 2); pharyngeal skeleton (Pl. II, fig. 6) 0.46 mm. long, shaped somewhat like that of *H. ascita* but more extensively pigmented, opaque black pigment continuous from cheliform spot back to dorsoventral fork.

Notiphila loewi Cresson (Ephydridae)

Several European authors, including Muller (1922), Varley (1937), and Hennig (1943), have contributed to knowledge of the biology and metamorphosis of *Notiphila*. It seems that larvae and pupae of most species live in the soil in bottoms of lakes, ponds, and streams and have sharp, hollow terminal spines by means of which they obtain oxygen from the intercellular gas spaces of aquatic plants.

Apparently very little is known about the immature stages of *Notiphila* in this country. From statements of Goureau (1851), Needham, Frost, and Tothill (1928) drew the general inference that the larvae are miners in leaves of plants. However, the literature shows that most *Notiphila* larvae of known habits are not leaf miners, and it may be that the genus contains no leaf-mining species. Marchal (1903), Muller (1922), and others have pointed out that the flies which Goureau reared from leaf mines in nasturtium and identified as *Notiphila flaveola* Meigen actually belong to the genus *Hydrellia*.

Johannsen (1935) omitted *Notiphila* from his key to larvae and puparia of aquatic Ephydridae and wrote, "Though the larvae of at least some members of . . . Notiphila . . . are aquatic, they either have not been described at all or have not been described in sufficient detail to be included in the above key. The eggs of Notiphila are laid on water plants."

Cresson (1944, 1946), who presented biological notes on many of the Ephydridae, discussed (1946) 21 species of *Notiphila*, but ventured no statement regarding their biology and immature stages. Concerning *N. loewi*, he write, "A relatively common species from Canada, Maine, New Hampshire, Michigan, Minnesota, Ohio, Illinois, Tennessee, Florida, Wyoming, Colorado, Utah, and Washington."

In the present study, a few larvae and many puparia of *N. loewi* were found attached to the roots of *P. alpinus* and *P. Richardsonii* in several localities in Cheboygan County, Michigan. Puparia were also found on roots of *P. pectinatus* in Cheboygan County.

Egg and immature larva were not observed.

Mature Larva (living) (Pl. IV, fig. 1).—Elongate; cylindrical, tapered toward both ends; about 8.0 by 1.2 mm.; light gray; segments not distinct; no creeping welts apparent; metapneustic with posterior spiracles in the form of slits near the tips of two hollow terminal spines; anterior end with paired, three-segmented antennae and paired inferior tubercles; pharyngeal skeleton (Pl. IV, fig. 2) composed of pharyngeal and hypostomal sclerites, colored as shown and bearing two mouth hooks; pharyngeal sclerite about 0.64 mm. long, with lateral halves connected together by anterodorsal bridge and both dorsal rods forked posteriorly.

Puparium (Pl. IV, fig. 3).—Cylindrical and tapered more gradually to posterior than to anterior end; transparent brown; 4.6–5.1x1.4–1.7 mm.; terminal respiratory spines conspicuous, about 0.18 mm. long; anal plate extending around approximately one-half the circumference of puparium, and bearing anus as midventral, longitudinal slit; enclosed pharyngeal skeleton as figured and described for larva; living pupa at first light colored, later dark brown, with red compound eyes conspicuous.

Nothing was observed to indicate that larvae of *N. loewi* feed on *Potamogeton* tissues. Form of the respiratory spines and the fact that larvae and puparia were found thus attached to roots suggest that these flies may be dependent on plants for oxygen during their larval and pupal stages. It is noteworthy that these immature stages live in black humous bottoms which are rich in decaying organic matter and practically devoid of free oxygen. Embedded in this material and thus isolated from circulating water, these insects have no apparent source of oxygen except from the roots of plants.

All attempts to rear these flies by isolating puparia together with small segments of attached roots failed, even when the pupae within were well matured in nature. If floated in water, the fine roots quickly became waterlogged, and the puparia filled with water and sank. If placed in moist atmosphere, the roots wilted, collapsed, and presumably admitted no more oxygen into the puparia. It would be difficult to transplant entire plants into screen-covered aquaria without breaking the brittle puparia or the delicate roots to which they are attached, and impossible to associate each fly which emerged in such a large container with its empty puparium. This problem was finally solved by carefully teasing all root tissue from a few puparia and placing them

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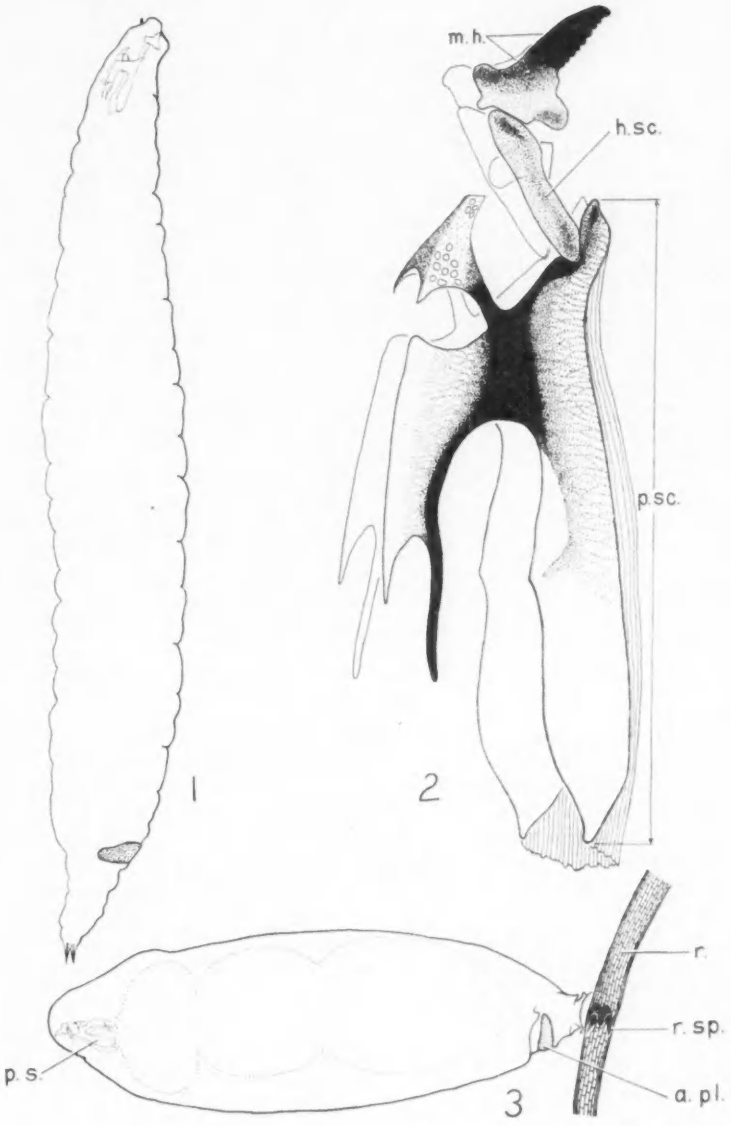
EXPLANATION OF PLATE IV

Immature stages of *Notiphila loewi* (Ephydridae). FIG. 1. Lateral view of larva. FIG. 2. Pharyngeal skeleton and mouth hooks. FIG. 3. Lateral view of puparium, attached to root. h. sc., hypostomal sclerite; p. sc., pharyngeal sclerite, showing total length; r., root of *Potamogeton*. Other abbreviations as in Plate I.


m.h.
h.sc.
p.sc.
1
2
r.
r. sp.
a. pl.
p. s.
3

in moist sphagnum moss, each in a separate vial, with respiratory spines projecting up into the air.

Hydromyza confluens Loew (Cordyluridae)

Studies on *Hydromyza confluens* by Needham (1908) and Welch (1914, 1917) have revealed that its normal food plant is the yellow water lily. They reported that the larva lives within lily petioles, feeds on petiole tissues, and produces gall-like swellings. Pupation occurs within the cavity excavated by the larva, the puparium being completely covered by plant tissue.

A puparium which I found attached by its posterior respiratory plates to the roots of *Potamogeton alpinus* in Nigger Creek, Cheboygan County, Michigan, June 25, 1946, was detached from the root and placed on moist cotton in a rearing bottle. The freshly emerged male imago was discovered 13 days later, at 7:37 a. m. After this specimen had stroked the wings and body, stamped around, and rubbed the tarsi together for 14 minutes, the wings expanded rather suddenly. This specimen, dry and in very good condition, was killed and pinned two days later, and has been identified as *Hydromyza confluens* Loew by Fred M. Snyder, of Orlando, Florida.

Except for the posterior respiratory plates, which were covered by plant tissue, the major portion of this puparium projected into the black humus and silt of which the bottom is composed in a manner similar to that of *Notiphila loewi* (Pl. IV, fig. 3). It is evident that the respiratory plates were well embedded in plant tissue because the puparium remained attached to the root and was pulled up through the bottom soil with the plant. Under these unusual circumstances, development apparently had proceeded normally.

Summary

1. Biological data on eight species of acalyptrate flies reared from *Potamogeton* are recorded, and immature stages of seven of them are described and figured.

2. A key is presented to six species of *Hydrellia* (Ephydridae) which mine in leaves and stems of *Potamogeton*.

3. Immature stages of *Hydrellia ascita*, *H. bergi*, *H. caliginosa*, *H. cruralis*, *H. luctuosa*, and *H. pulla* are depicted, all except one of them for the first time.

4. Activities of *Hydrellia* larvae and adults, life cycles, seasonal aspects, and modes of hibernation are discussed in some detail; food plants and parasites are listed.

5. Data are presented on the biology of *Notiphila loewi* (Ephydridae), larvae and pupae of which live in humous bottoms with respiratory spines inserted in *Potamogeton* roots; descriptions and figures of immature stages are given.

6. Normal development of a *Hydromyza confluens* (Cordyluridae) pupa in the silty, humous bottom of a stream with respiratory plates embedded in a *Potamogeton* root is recorded.

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MEDICAL ENTOMOLOGY, by ROBERT MATHESON. vi+612 pages, 242 figures, 4 plates. Second edition. Comstock Publishing Company, New York. 1950. Price, $7.50.

A major handicap in the teaching of medical entomology has been the lack of a textbook which adequately presents the more recent developments in the subject. This revision of a leading prewar text by an outstanding medical entomologist will, indeed, be welcomed both by students and teachers of the subject. The author's experience and familiarity with culicidology has aided greatly in the assimilation of the immense amount of literature which it has been necessary to review, since such an important part of that literature deals with the mosquitoes; however, his grasp of the entire, complex subject has led to the production of a textbook of high quality.

The treatment is for the most part traditional. An introduction paves the way to an understanding of the problems involved and gives a brief historical background for understanding the Acarina, Insecta, and other more important arthropods involved in the transmission and production of human disease, after which he discusses the biologies, role in disease transmission and production, the diseases involved, and control and prophylaxis. Veterinary aspects are included only incidentally. In general, a taxonomic order is maintained, although the Acarina are widely separated from the poisonous Arachnida; this may not be wholly logical, since certain ticks might be discussed under both headings. The account of Diptera, as would be expected, takes up more than half the text (pp. 218–537). Keys and tabular material are employed freely. A final chapter deals with collecting and preserving methods.

The book is well-written, with well-chosen illustrations which are for the most part well-executed. One can find but little to criticize; a few things might, however, be mentioned. The author's statement that nothing is known of the biology of *Wohlfahrtia opaca* is not correct; at least ten papers, including two by the reviewer, have been published on this subject. The horrible illustration in fig. 186 is somewhat misleading, since in this case the screwworm attack was secondary and consequently not responsible for all the injury; a more typical illustration might have been used. Hall's "Blowflies of North America" is omitted from the references to Chapter XVII, although the terminology of the Calliphoridae is mainly adapted from that work. In this connection, it is noteworthy that Dr. Matheson does not recognize the generic name *Phaenicia*, although that genus is recognized as valid not only by Hall but also by such dipterists as Curran, Townsend, and Malloch. It is only fair to say, however, that certain leading dipterists do not accept that genus. In matters of terminology, Dr. Matheson is conservative, though sometimes inconsistent. This conservatism is also evident in his discussion of control measures, where it is definitely desirable, in view of the rapid advances in this field which are constantly making yesterday's recommendations obsolete today.—M. T. J.

A NEW SPECIES OF ORTHOPODOMYIA FROM CALIFORNIA (Diptera, Culicidae)[1]

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The California representative of the genus *Orthopodomyia* has previously been confused with *signifera* (Coquillett), a widespread species of eastern United States west to Texas and Nebraska. Considering the geographical separation involved, it is not surprising that the Californian form has characters indicating that it is a distinct species.

Differences observed in the adults and pupae of the three United States species are minor but larval differences are well marked. The three species can be separated in the larval stage according to the following key.

KEY TO ORTHOPODOMYIA OF AMERICA NORTH OF MEXICO BASED ON FOURTH STAGE LARVAE

1. Siphon tuft less than three-fourths (0.47 to 0.68) as long as length of siphon[2] beyond insertion of tuft (fig. 2); tuft with 2 to 5 branches; lateral hair of anal segment divided from base into 2 to 4 branches, longest branch about as long as or longer (0.97 to 1.65) than length of siphon before insertion of tuft; no sclerotized plate on abdominal segment VIII; transutural hair of head (hair 9) strong and multiple..... **alba** Baker
   Siphon tuft more than three-fourths as long as length of siphon beyond insertion of tuft; tuft with more than 4 branches; lateral hair of anal segment single or divided beyond base, less than three-fourths as long as length of siphon before insertion of tuft; a sclerotized plate present on abdominal segment VIII; transutural hair weak and single, often frayed toward apex..... 2
2. Siphon tuft about as long (0.81 to 1.16) as length of siphon beyond insertion of tuft (fig. 3)..... **signifera** (Coquillett)
   Siphon tuft more nearly twice as long (1.72 to 2.26) as length of siphon beyond insertion of tuft (fig. 1)..... **californica** n. sp.

Orthopodomyia californica, new species

Female.—Length of wing 4 mm. Vertex with narrow curved white scales in median area and along eye margins, a lateral spot of broader white scales, upright forked scales numerous and black; torus with an inner patch of white scales; palpus and proboscis with numerous white scales above, latter about three times as long as former. Thoracic integument dark brown to black; scutum with many black bristles, and with three pairs of longitudinal hair-like silvery lines, outer pair extending entire length of scutum, middle pair extending forward to middle and broken halfway, inner pair extending back two-thirds of scutal length, scutellum with black bristles but devoid of scales except for a continuation of middle scutal lines; pleuron with a line of white

[1]Cost of publication paid in part by the University of California.

[2]Length of siphon taken as greatest length of sclerotized area but excluding apical valves.

scales across anterior pronotal lobe and posterior pronotal lobe, a broad line of scales from propleuron across sternopleuron and mesepimeron, a patch on lower sternopleuron, and a few other pale scales; wing with rather broad, scattered white scales, concentrated into spots near middle of wing (on veins 4 and 5.2), at base of vein I and basal two-fifths of vein 6; halter knob pale scaled; femora and tibiae speckled black and white, tibiae lined in front; front and mid tarsi with small or indistinct, pale, joint spots, hind tarsus with distinct joint bands and all of V white above, joint band of I-II about equal in length on both segments and whole band shorter than segment IV; joint band of II-III a little shorter than segment V, joint band of III-IV about one-half as long as segment V. Abdominal segments dark with narrow pale basal bands on III to VI, II pale medially and with a lateroapical dark area, I with a medioapical pale spot; venter with basal segmental pale spots.

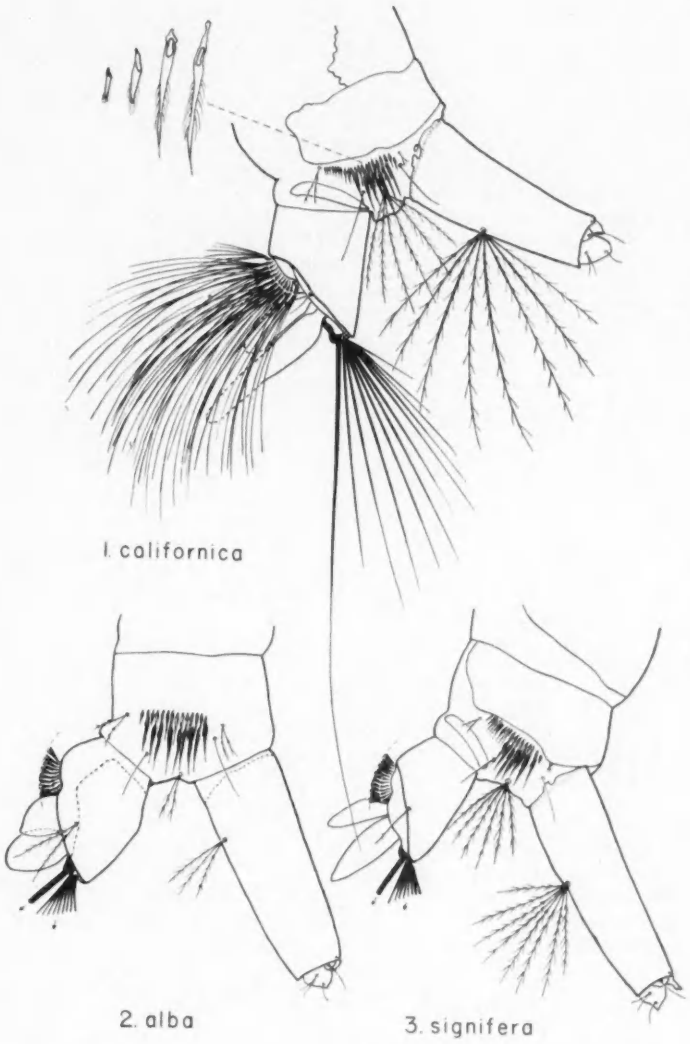
Male.—Palpus about as long as proboscis, a line of pale scales above on basal four-fifths of long segment, base of subterminal and all of minute terminal segment white. Antenna dark with brownish reflection.

Larval skin of type (fig. 1).—Antenna smooth, a three-branched, frayed tuft inserted at basal one-third and reaching beyond apex of shaft; clypeal spine long and slender, head hair A with 6 to 8 branches, B with 7 to 8 branches, C with 6 to 7 branches, d with 5 to 6 branches, e and f single; mentum with 17 teeth, the basal three largest. Shoulder hair 1 double, 2, 5 and 6 single, 3 triple, 4 quintuple, 7 with 7 branches; mesonotal submedian hair triple, metanotal submedian hair single. Lateral hairs of abdominal segments I and II quintuple; a small dorsal plate on VI, large ones on VII and VIII, comb with 21 teeth in upper row and 7 in lower row, other characters as shown in fig. 1. Siphon tuft a little more than twice as long as length of siphon beyond point of insertion, eight-branched (5 to 9 branches in paratypes), located slightly nearer apex in some paratypes; lateral hair of anal segment inserted at edge of plate, slender, short.

Pupal skin of type.—Apicolateral tuft of segment VII with 4 to 6 branches (3 to 8 in paratypes); apicolateral tuft of segment VIII with 9 to 10 branches (7 to 12 in paratypes); paddle hair single to double, frayed toward apex, one-seventh as long as paddle.

Type, female and its larval and pupal skins, near Sacramento at Elkhorn Ferry, Yolo Co., California, April 12, 1948, from cottonwood tree-hole (R. M. Bohart), deposited in U. S. National Museum. *Paratypes*, 65 males (22 with associated skins), 57 females (10 with associated skins), and 49 fourth-stage larvae, same data as type except that some were collected August 26, 1948, and others on November 3, 1949. Larvae were also studied from Riverside, California, May 17, 1948, from cottonwood treehole (W. C. Reeves), and Bakersfield, California, May 17, 1948, from cottonwood treehole (E. Hill).

Distribution.—The occurrence of *Orthopodomyia* in California was first noted by Ludlow (1906) who reported "*Culex* (?) *signifer*" from Benicia Barracks, Solano County. The second published record was that of Reeves (1941) who recorded the capture of large numbers of larvae and pupae from a treehole in a cottonwood in Riverside, Riverside County, and cottonwood and willow treeholes near Redlands,



Terminal segments of fourth instar larvae. FIG. 1. *Orthopodomyia californica*, cast skin of type. FIG. 2. *O. alba* from Williamsburg, Virginia. FIG. 3. *O. signifera* from Williamsburg, Virginia.

San Bernardino County. Subsequently, *Orthopodomyia* have been collected either in the adult or larval stage near Bakersfield, Kern County (G. F. Smith and E. Hill), near Hanford, Kings County (Willis Wirth), at Yolo, Yolo County (E. G. Meyers), at Nice, Lake County (A. W. Lindquist), and by the writer near Elkhorn Ferry, Yolo County. It seems likely that this mosquito is widely distributed in California wherever suitable rotholes in cottonwood or willow are found.

Biology.—The Elkhorn Ferry collections were made in April, May, and August of 1948 and in November and February of 1949. Second to fourth stage larvae were taken in April and May, various larval instars and pupae in August, first to fourth instars but no pupae in November, and first to fourth instars but no second or third instars or pupae in February. From these observations it can be surmised that there are several overlapping generations through the summer and that overwintering takes place in both the fourth larval instar and the egg.

An association with cottonwood seems to be characteristic of *californica*. At the Elkhorn Ferry locality larvae of both *californica* and *Aedes varipalpus* (Coq.) were collected during April, May and August in 15 cottonwood treeholes, while in the same area 12 oak treeholes yielded only *varipalpus*. However, the specimens from Nice, cited above, were from oak treeholes.

The larvae vary in color from greenish to pink to purple presumably depending upon the amount of the pigmented bacterium, *Thiocaspa* ingested. Reeves (1940) found that pigmented larvae lost their color when changed to a yeast diet but regained the color when put back into the natural medium. It is interesting to note that the cottonwood treehole water in the Elkhorn Ferry locality has a pH of 8.0 to 8.5.

The aquatic stages of *californica* develop more slowly than any other species in the west, the nearest competitor being *Aedes squamiger* (Coquillett). First stage larvae collected on February 16, 1949, and kept in the laboratory in treehole water on a diet of powdered dog food reached the fourth instar in June, a period of about 4 months. Pupation took place late in June and the pupal stage lasted 7 to 8 days instead of the 3 or 4 days usual for most species of mosquitoes.

The ability to live for long periods on a poor diet was shown by three early fourth stage larvae which were collected August 26, 1948, and maintained in the laboratory in a glass-covered dish in diluted treehole water and no supplemental ration. One larva lived 7 months, another 8 months and the third 9 months. The first two died before pupation but the third pupated on May 26, 1949, and died 3 days later.

Systematics.—The genus *Orthopodomyia* contains about a dozen described species of essentially treehole-breeding mosquitoes from the Oriental, Holarctic, Ethiopian and Neotropical Regions. On the basis of scutal markings they can be divided into two groups, first with markings patchy, and second with longitudinal silvery lines. In the first group are *fascipes* (Coquillett) from Central and South America, *arboricollis* d'Emmerez de Charmoy from the island of Mauritius, and the Oriental species, *albipes* Leicester (type of genus), *flavithorax* Barraud, and *flavicosta* Barraud. The species with silvery scutal lines include *pulchripalpis* Rondani from Europe; *phyllozoa* (Dyar and

Knab) and *albicosta* Lutz from Central and South America; and *signifera* (Coquillett), *alba* Baker, and *californica* from the United States.

In the second group the three exotic species have characteristic wing markings which separate them from each other and from the three United States species which have a common distinctive wing pattern. *O. californica* is most closely related to *signifera*. In the adult the male genitalia appear to be identical and all other adult characters are very close. However, *signifera* specimens studied have a larger white ring over the joints between hind tarsal segments I-II and II-III, with the ring covering a greater length of I than of II and II than of III in both sexes. In *californica* the ring usually has about an equal length on I and II, and II and III in the female. The male is not always so definite in this respect. This character has been checked with U. S. National Museum specimens by Alan Stone who reports (in letter) that the difference is significant. He also remarks that in *alba* "the bands are as broad as in *signifera* but they are more evenly placed on the joint . . . " The difference is such that "it (*alba*) can be separated from *californica* without trouble, but it would be more difficult to separate it from *signifera*." Perhaps an exhaustive study of adult *alba* will reveal more definite points of distinction.

Characters of the pupa are also minor and somewhat variable. Based on 25 specimens from near Sacramento and Bakersfield, the paddle hair of *californica* is usually single but sometimes double. In either case it is frequently frayed toward the apex and may be split into several branches a short way from the base. In the eight specimens studied of *signifera* from Ames, Iowa, and New River, North Carolina, the paddle hair was rarely double, triple or sextuple, and usually quadruple or quintuple, the branches coming from the base of the hair. In two specimens supposedly of *alba* from Camp Crowder, Missouri, the paddle hair was double, triple, quintuple and sextuple, thus falling within the range of *signifera*.

In contrast with the other stages there is a marked difference between the larvae of the three United States species. This is all the more remarkable because of the similarity among larvae of several species of the genus which have obvious adult differences. Thus, *pulchripalpis* larvae are hardly separable from those of *signifera* even though the adult wing and proboscis markings are clearly distinguishing features. Both *fascipes* and *albipes*, which belong in the group with patchy adult scutum, have larvae differing only slightly from that of *signifera*. It is interesting, therefore, to find the reverse situation of almost identical adults and distinct larvae in the United States species.

In comparing the eastern species, Jenkins and Carpenter (1946) state that "It is possible that *O. alba* is a genetic variant, or that it is a well marked and distinct extreme of the natural variation of *O. signifera*." This idea, which could apply equally well to *californica*, seems unlikely in view of the stability observed in other species of the genus, and the rather small amount of variation noted within *alba* and *californica* populations.

Good series of larvae of *albipes* from the Philippine Islands and of *fascipes* from the Canal Zone were furnished by E. S. Ross of the California Academy of Sciences for a study of variation in exotic species in

comparison with domestic forms. As noted above, these two species resemble *signifera* in the larval stage. In *albipes* the siphon is more slender toward the apex and in *fascipes* the most obvious difference lies in the greater development of the thoracic hairs with the submedian mesonotal tuft many-branched rather than single. In these and other anatomical features it was found that the two species were very constant. This stability is one of shape, especially of the siphon and relative development of hairs, rather than exact number of hair branches and extent of sclerotized plates on abdominal tergites. It has been pointed out by Marshall (1938, p. 261) and Reeves (1941) that the sclerotized dorsal plates are increased in extent and number during the fourth stadium. Breland (1947) has discussed variation in *alba* and has shown that the size of the anal ring is subject to variation and may be complete, thus invalidating a character previously used in separation. However, many other relatively constant features remain for differentiation. These are discussed by Schoof and Ashton (1944).

In comparing larvae of the three United States species various characters were tabulated for the 17 available specimens of *alba* from Williamsburg, Virginia, and Camp Crowder, Missouri;[3] a like number of specimens of *signifera* taken at random from Virginia, North Carolina, Florida, Louisiana, Texas and Iowa; and 17 *californica* selected at random from Riverside, Bakersfield and near Sacramento, California. The obvious differences in the siphon and anal segment in the three species can be seen in figures 1–3. The differences and variations within species are best expressed as ratios. Thus the length of the siphon tuft divided by the siphon length posterior to insertion of the tuft averaged 0.55 (range 0.47–0.68) for *alba*, 0.96 (range 0.81–1.16) for *signifera* and 2.00 (range 1.72–2.26) for *californica*. Many additional specimens of *signifera* and *californica* were examined and found to fall respectively within the above ranges. Similarly, the length of the lateral hair of the anal segment divided by the siphon length anterior to the siphon tuft averaged 1.25 (range 0.97–1.65), for *alba*, 0.54 (range 0.35–0.67) for *signifera*, and 0.42 (range 0.33–0.61) for *californica*. The latter ratio distinguishes *alba* but not the other two species.

Institutions and individuals who cooperated by lending material were the U. S. National Museum (through Alan Stone), California Academy of Sciences (through E. S. Ross), S. F. Bailey, W. C. Reeves, and Jean Laffoon.

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[3]Camp Crowder specimens were borrowed from the U. S. National Museum.

THE WEST INDIAN SPECIES OF PHLEBOTOMUS

(Dipt. Psychodidae)

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Sandflies of the genus *Phlebotomus* are a widespread and medically important group of bloodsucking midges. Their distribution covers the tropics of both hemispheres and extends well into both the north and south temperate regions. Certain species are the vectors of various forms of leishmaniasis, of Oroya fever or verruga peruana and of pappataci or sandfly fever. Leishmaniasis in several forms is widespread in the American tropics, and verruga is known from southern Colombia to southern Peru, but neither infection has been reported from the West Indies. Sandfly fever is known with certainty only from the Old World, though cases clinically indistinguishable have been reported from a number of widely scattered places in the New World. Clinically suspicious cases have occurred in Puerto Rico, though not reported as sandfly fever because of the supposed absence of *Phlebotomus* in that area.

Previous to 1938, *Phlebotomus* sandflies were not known to occur in the West Indies outside of Trinidad. In that year Bequaert (1938) reported their occurrence in Martinique in the French West Indies, although the species has not been determined. Floch and Abonnenc (1945) were the first to determine a species from the West Indies, *P. atroclavatus* from Guadeloupe. In 1947 the junior author took *Phlebotomus* on Puerto Rico and on Vieques Island. These were described by Fairchild and Hertig (1948). In the following year Trapido again secured material from a number of widely scattered localities in Puerto Rico and from St. Thomas in the Virgin Islands which is reported on more fully below.

[1]The field work in connection with this investigation was done while the authors were on tour of active duty with the U. S. Army Caribbean. We are exceedingly indebted to Col. Thomas Page, M.D., Surgeon, U. S. Army Caribbean, for his interest and support of this work and for facilitating the arrangements for our trip. We are further greatly indebted to Mr. C. B. Lewis of the Institute of Jamaica for much help and advice while we were in Jamaica; to Col. Carlos Ponce R., of the Cuban Army, for his hospitality and kindness in furnishing quarters and transportation while we were in Camaguey; to Dr. Guillermo Aguayo and Lt. Howell of the University of Havana and the Cuban Hydrographic Service for enabling us to collect in the Havana area; to Major E. L. Dudley of the Institute of Inter-American Affairs Sanitary Mission in Port au Prince and to Mr. John Lynch of the Reconstruction Finance Corporation in Cap Haitien for furnishing us with good advice and indispensable transportation, and finally to Dr. Henry Carr, of the International Health Division of the Rockefeller Foundation, who very kindly furnished us with a car and driver for our whole stay in the Dominican Republic. Without the material aid furnished by these gentlemen our trip would have been very much more difficult and much less productive.

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The discovery of these potentially important insects in the West Indies made it seem advisable to find out if they were generally distributed in the area, especially on the larger islands, and, if present, likely to become of public health significance. It was felt that the question was one of special interest to the Army, due to the strategic position of the West Indies and the previous experience of our forces with sandfly fever in the Mediterranean area. Accordingly arrangements were made for the authors to return to active duty for 30 days in order to accomplish the field work.

Field work was begun on 19 May 1949, in Jamaica, where nine days were spent. Search was made for *Phlebotomus* in approximately forty localities in eight of the thirteen parishes of the island. *Phlebotomus* were found at thirteen localities in six different parishes at elevations from sea level to about 1300 ft. Searches at higher elevations up to 4200 ft. were negative. A total of 97 specimens was taken, but at only a few places were sandflies abundant. We believe they will be found throughout the island at lower elevations, especially where suitable tree habitats occur.

The time from 28 May to 5 June was spent in Cuba. Search was made in four of the six provinces, Camaguey, Matanzas, Habana and Pinar del Rio. Because of the limited time available and the large size of the island more time was consumed in travelling and less in collecting than in Jamaica. It was impossible to visit the eastern end of Cuba, though the senior author had searched the desert area around Guantanamo Bay in 1946 without result. About twenty localities were searched in Cuba, from sea level to about 1000 ft. *Phlebotomus* was found at thirteen localities in six different parishes at elevations specimens was taken, 132 of these at one locality near Camaguey, the only time we found sandflies really abundant. At this place, Finca Chiquito Ingenio, the insects were hopping about on the trunks of mango, breadfruit and sapodilla trees in an overgrown orchard. Domestic swine shared the enclosure with chickens, turkeys and peacocks, while lizards were very abundant. Only *P. cubensis* n. sp. was taken here. At Finca El Milagro, where habitats in the form of deep buttresses and hollow trees were far more abundant, sandflies were very scarce, and we took but five specimens, two of them the new *P. orestes*, and all from the same large Ficus tree. Jointly we searched at least a hundred habitats in this patch of forest. In Western Cuba in the Vinales Valley sandflies were also scarce, a total of eight, all taken by Trapido, resulting from some 20 hours searching. They were confined to dense thickets along a stream, a single specimen here and there. Searches of limestone cavities in the sheer cliffs characteristic of this area or in the open and rather xerophytic oak-pine forests yielded nothing.

We were from 6 June to 15 June in Hispaniola, four and a half days in Haiti, and five days in the Dominican Republic. Thirty-eight localities were searched, including both north and south coasts and the highlands up to 5200 ft. elevation. Sixteen localities yielded *Phlebotomus* at elevations up to 1900 ft. A total of 162 sandflies was taken on the island, 98 in Haiti and 64 in the Dominican Republic. Near Port au Prince in buttresses of a large Ceiba tree we secured some 30

specimens, mostly females, and all of them engorged with blood. Again near Cap Haitien some 30 specimens were taken in a patch of old second growth, heavily shaded, in the narrow cracks in the trunks of logwood trees. Here too the majority of females were engorged. The ruins of King Christophe's palace of Sans Souci, at Milot near Cap Haitien and Pauline Bonaparte's old palace on the outskirts of the town yielded fair numbers of *Phlebotomus* from crevices and holes in the old masonry, the only place on the trip where we found these habitats rewarding. In the Dominican Republic our best collecting was in association with trees, either large roadside Ceibas, or shade trees in coffee or cacao plantations, but nowhere were sandflies very numerous. Our single venture into the highlands yielded nothing, though we found a fine small patch of apparently virgin deciduous forest with many suitable habitats including small animal burrows. This locality was at over 5000 ft. in mainly pine forest.

Because of the very limited time available we felt that a rapid reconnaissance of as many localities and types of habitat as possible was likely to yield the best results. For this reason we concentrated on searches of daytime resting places likely to harbor sandflies and made no attempts at trapping, light collecting, house searches or the use of animal baits. Suitable daytime resting places for these insects consist of animal burrows, hollow trees, crevices between the buttressed roots of large trees, caves and crevices in and under rocks, holes in masonry walls and, in general, small cavities which are dark and humid. Since the West Indies are extremely poor in mammals and have been very extensively deforested, we expected our best collecting to be in rock crevices and holes in masonry walls. We found, however, that the crevices between the buttressed roots of trees yielded the most sandflies. In Jamaica the large silk cotton trees (*Ceiba* sp.) are believed to be the chosen abode of "duppies," an endemic form of ghost, and are hence seldom felled. Since practically all the other large trees have long since been destroyed and the silk cotton trees, especially when old, have large buttressed roots, they proved one of the most suitable habitats for *Phlebotomus* on all three islands, but especially in Jamaica. In general, habitats associated with trees seemed the most favored, though we took sandflies in rock crevices and holes in masonry walls a number of times. Animal burrows were encountered only once, in Hispaniola, and yielded no sandflies.

Collecting was done with the aid of cigarette smoke and a suction tube. Smoke was blown into the habitat and the sandflies, if any, taken up in the suction tube as they emerged. If the hole or crevice was especially dark, a flashlight was used. The sandflies were killed in chloroform tubes and transferred to dated and numbered dry shell vials. This method of collecting has proven efficient elsewhere, and enabled us to sample a maximum of localities in a minimum time. At the same time we secured a fair number of mosquitoes which are being studied and will be reported on by our colleague Mr. Pedro Galindo.

We encountered no evidence that the species of *Phlebotomus* in the West Indies feed on man. We ourselves were not exposed, but we found no one who recognized them and no local names for them, a

condition in marked contrast to that on the mainland, where many native names occur. It is also significant that, although many competent entomologists have visited or worked in these islands, including medical entomologists searching for biting insects, ***Phlebotomus*** has remained undetected until now. Many of the females we took contained red blood, so that some vertebrate is doubtless the host, most probably lizards, as they are the most numerous terrestrial vertebrates in the West Indies and favor similar habitats.

The material collected shows the presence of two species on each of the three islands visited. In the case of Cuba, both species appear to be new and endemic, while Hispaniola and Jamaica each have one

TABLE I

MEASUREMENTS IN MICRA

	jamaicensis		*hispaniolae*		*duppyorum*		*cubensis*		*orestes*		*christophei*	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
Ant. III	212	196	228	164	324	240	216	172	200	168	248	216
Palpi I+II	140	112	112	88	156	116	148	96	128	120	160	116
Palpi III	136	120	120	104	168	120	140	104	124	120	144	120
Palpi IV	108	76	92	76	116	80	88	80	92	84	100	88
Palpi V	308	240	284	200	368	268	280	180	296	240	336	260
Head ht	204	196	220	168	208	200	220	188	220	220	260	208
Clypeus	124	112	128	100	120	100	120	100	120	112	148	104
Proboscis	220	160	160	120	180	144	172	120	172	164	240	144
Eye ht	148	136	152	128	144	136	168	140	132	120	168	124
Wing	1746	1440	1656	1224	2016	1566	1710	1350	1476	1350	1854	1458
Alpha	468	324	450	288	504	288	378	216	216	180	450	288
Beta	306	252	270	180	378	306	342	252	216	198	270	198
Gamma	230	180	234	180	324	216	216	198	288	288	288	234
Delta	306	198	306	162	288	72	234	90	18	—18	162	18

peculiar species and one species probably only subspecifically distinct from the form found in Puerto Rico. The endemic Jamaican species and one of the Cuban species belong to the same group as the Puerto Rican species, but are abundantly distinct. The other Cuban species and the endemic Hispaniolan species are closely related to each other but belong to a quite different group from the other Antillean species. Including *atroclavatus*, there are thus three groups represented, all of which either occur on or have representatives on the mainland. In the case of *cayennensis*, the most widespread form, there are poorly marked subspecies on Puerto Rico, Vieques, Virgin Islands, Hispaniola and Jamaica as well as in Mexico, Panama and northern South America. *P. duppyorum* n. sp. and *P. cubensis* n. sp. also belong to the *cayennensis* group, but have developed characters sufficiently marked to be considered full species. *P. orestes* n. sp. and *P. christophei* n. sp. have close relatives in Panama and northern South America, while *P. atroclavatus* is known from Panama, Trinidad and French Guiana.

The Table of Measurements does not separate the sexes nor are means calculated, as too few specimens were available in most cases to make this of any importance. Usually the maximum figures occurred in females, the minimum in males, but no single specimen was smallest or largest in all measurements. On the whole, the measurements appear of little value in separating these species, though the long third antennal of ***duppyorum*** and the relatively shorter delta in ***orestes*** and ***christophei*** may be useful.

KEY TO WEST INDIAN SPECIES

Males

1. Coxite with a basal tuft of hairs 2
   Coxite without a basal tuft of hairs; style with four major spines 4
2. Style with 4 major spines; basal tuft of 4 heavy setae ..... **atroclavatus**
   Style with 3 major spines and an accessory subterminal seta 3
3. Basal tuft of numerous fine hairs borne on a salient rounded prominence; tips of genital filaments modified and expanded ..... **christophei**
   Basal tuft of two groups of heavier hairs, less numerous and not borne on a prominence; tips of genital filaments little modified and hardly expanded, **orestes**
4. Cibarium with hardly visible vestiges of teeth, few in number; pharynx slender; third antennal segment longer than clypeus and proboscis; genital filaments slender ..... **duppyorum**
   Cibarium with rather well marked teeth or spines; pharynx stouter; genital filaments stouter; third antennal segment considerably shorter than clypeus and proboscis 5
5. Cibarium with 6 spine-like horizontal teeth; no erect teeth ..... **cubensis**
   Cibarium with more than six horizontal teeth and few to many erect teeth ..... **cayennensis** and subspecies

Females

1. Spermathecae with a thick, annulate common duct, the two spermathecae without individual ducts, tapering from a swollen wrinkled base to a slender tubular apex; cibarium with 4 long teeth; pharynx unarmed, **christophei**
   Spermathecae with individual ducts many times longer than the common duct, the latter slender 2
2. Heads of spermathecae pear-shaped, smooth, the ducts smooth; cibarium with 4 very small teeth; pharynx with strong transverse ridges and long spines ..... **atroclavatus**
   Heads of spermathecae subglobose, joining the ducts by a tapering, annulate portion; cibarium with 4 or more teeth in a comb-like row; pharynx spinose but without transverse ridges 3
3. Pharynx greatly expanded proximally, wine-glass shaped, finely spinose; cibarium with 6 to 8 short fine teeth, longest in the middle ..... **duppyorum**
   Pharynx and cibarium not as above 4
4. Cibarium with 4 straight teeth in a rudimentary comb; pharynx densely spinose at proximal end ..... **cubensis**
   Cibarium with 12–18 teeth in a comb-like structure; pharynx as above, **cayennensis** and subspecies

Phlebotomus christophei sp. nov.

Plate I, figs. 1 and 4; Plate II, figs. 3 and 7

Male.—A small pale sandfly without contrasting colors. Eyes relatively small, proboscis and palpi relatively long. Third antennal segment somewhat shorter than first three palpal segments. Ascoids simple, about two-thirds the length of their respective segments. Newstead's scales long and clubbed, in a large diffuse patch occupying the central third of the segment. Genitalia with three major spines

and an accessory subterminal seta, as figured. Genital pump and filaments about 1.25 times as long as coxite and style, the tips strongly modified, as figured. Cibarium without clearly visible teeth, the chitinous arch well marked but diffuse in the center. Pharynx unarmed.

Female.—External characters as in the male. Spermathecae as figured. Cibarium with four stout horizontal teeth and a number of small erect teeth below as figured. Chitinous arch high and heavily sclerotized. Pharynx unarmed. Cerci rather short and blunt.

This species is clearly related to *P. orestes*, of the present paper, from Cuba, but differs clearly in the structure of the basal tuft of the coxite as well as in other details. From the other American species of *Phlebotomus* with three spines on the style these two may be distinguished from *chassigneti* Floch and Abonnenc and *pilosus* Damasceno and Causey by the presence of well developed tufts of hairs on the base of the coxite. The species of Mangabeira's subgenus *Pressatia*, *P. triacanthus* and allies, are larger species, the subterminal spine of the style borne on a distinct tubercle, with an extra accessory seta on the style, and with more complex basal tufts on the coxite and more complex parameres. *P. vespertilionis* Fairchild and Hertig and *vesiciferus* F. and H. differ in having inflated lateral lobes.

Holotype male, slide 1476, 16 km. south of Hato Mayor, Prov. Seibo, Dominican Republic, 12 June 1949. In buttresses of large Ceiba tree.

Allotype female, slide 1497, Colonia San Rafael, 21 Km. south of Sabana de la Mar, Prov. Seibo, Dominican Republic, 900 ft. elev. in buttress of large tree in coffee plantation.

Paratypes, 4 males, 1 female, same data as allotype; 1 male, 1 female, Milot, nr. Cap Haitien, Haiti, 9 June 1949, in holes in wall of room in ruined palace of Sans Souci; 1 female, 34 km. south of Sabana de la Mar, Prov. Seibo, Dominican Republic, 650 ft., in buttress of large tree in coffee plantation. All Fairchild and Trapido colls. Named in honor of Christophe, King of Haiti, in whose palace the first specimens were taken.

Phlebotomus orestes sp. nov.

Plate I, fig. 2

Male.—A small pale sandfly without contrasting colors. Eyes relatively small, proboscis and palpi relatively long. Third antennal segment distinctly shorter than first three palpal segments. Ascoids simple, about half the length of their respective segments. Newstead's scales long and clubbed, in a small dense patch in the middle of the third palpal segment. Genitalia with three major spines and a subterminal seta on the style, as figured. Genital pump and filaments about one and one-half times as long as coxite and style, the tips not exserted in the available specimens, but apparently but slightly spatulate. Cibarium without visible teeth but with a well marked chitinous arch. Pharynx unarmed. Female unknown.

Holotype male and 1 male *paratype*, Finca El Milagro, between Minas and Altagracia, Camaguey Province, Cuba, 30 May 1949, Fairchild and Trapido colls. Taken in buttresses of a large tree in partly cut-over forest.

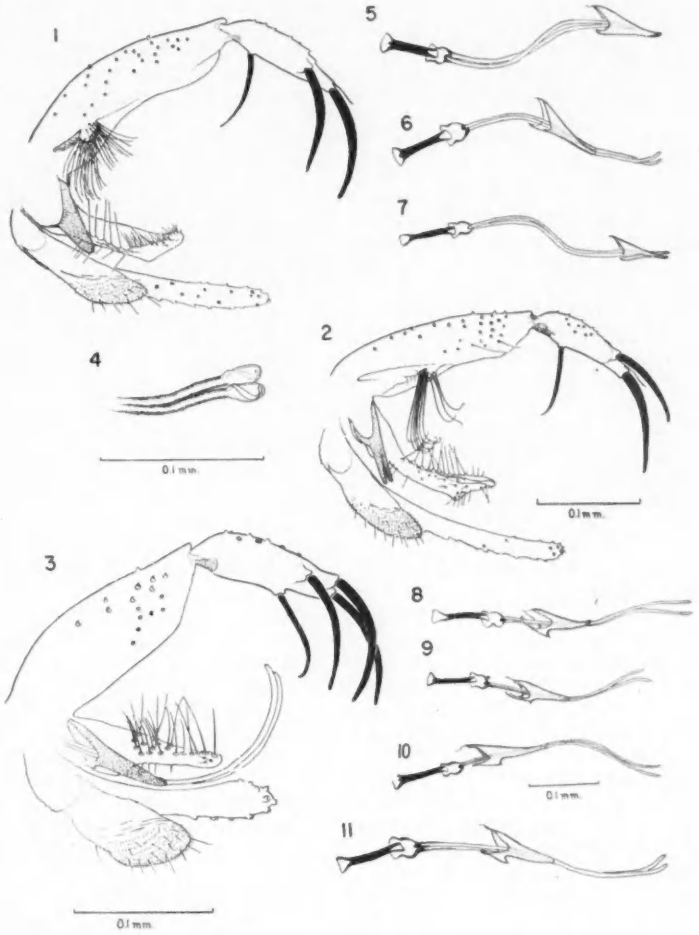


FIG. 1. *P. christophei* n. sp. Paratype, male genitalia. FIG. 2. *P. orestes* n. sp. Holotype, male genitalia. FIG. 3. *P. cubensis* n. sp. Paratype, male genitalia. FIG. 4. *P. christophei*, tips of genital filaments. FIGS. 5–11. Sperm pumps and genital filaments of: 5, *P. cayennensis viequesensis* (Virgin Islands); 6, *P. c. viequesensis* (Vieques Island); 7, *P. c. puertoricensis* (Puerto Rico), 8, *P. c. hispaniolae* n. subsp. (Dominican Republic); 9, *P. cubensis* n. sp. (Cuba); 10, *P. duppyorum* n. sp. (Jamaica); 11, *P. cayennensis jamaicensis* n. subsp. (Jamaica). Figs. 1 and 2 are to the same scale, indicated at fig. 2 and figs. 5–11 are all to the scale indicated at fig. 10.

This species seems closely related to *P. chrristophei* of Hispaniola and to an as yet undescribed species from Panama. From *christophei* it differs chiefly in the form of the basal tuft on the coxite and in the shape of the parameres. It also appears to lack the greatly enlarged tips to the genital filaments found in *christophei*, though since these are not exserted in our two specimens, close comparison is not possible.

Phlebotomus duppyorum sp. nov.

Plate I, fig. 10; Plate II, figs. 2, 8 and 9

Male.—A small slightly dusky sandfly with erect abdominal hairs. Eyes medium sized, third antennal segment slightly longer than first three palpal segments. Ascoids simple, about one-fourth the length of their respective segments. Newstead's scales scattered along the middle third of the segment. Genitalia with four major spines, differing from *P. cayennensis* only in the more slender and relatively shorter genital filaments. Cibarium with barely perceptible vestiges of teeth, the pharynx slender, unarmed.

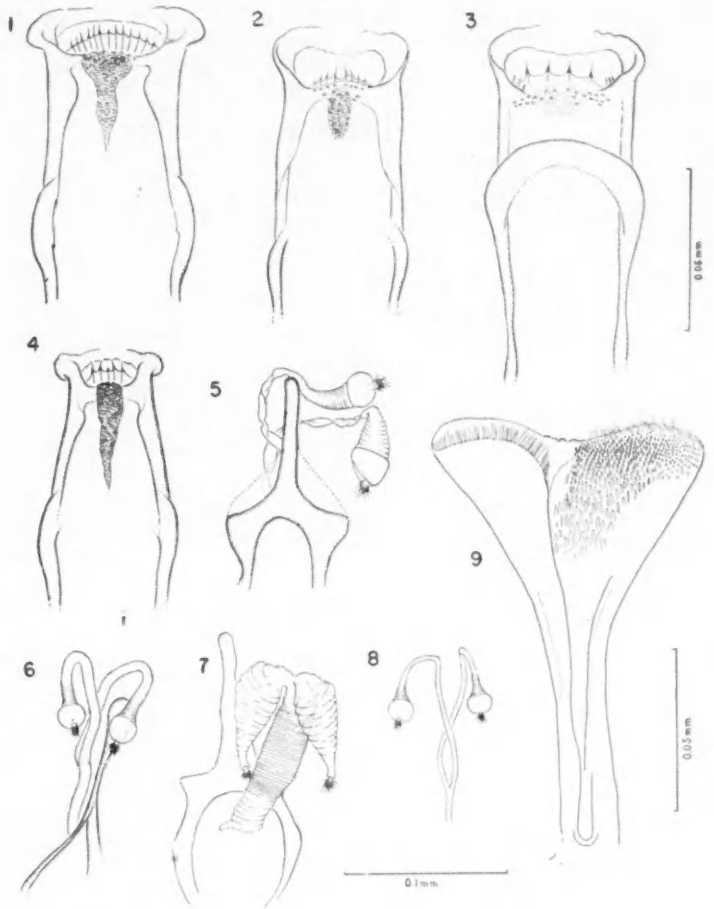
Female.—Externally like male, size slightly larger. Spermathecae as in *P. cayennensis*. Cibarium with 6 to 8 small teeth, longest in the middle, as figured. Pharynx greatly expanded proximally, wine-glass shaped, the proximal portion beset with numerous fine spines arranged in even diagonal rows.

Holotype, female, slide No. 1504, Ferry River, nr. Kingston, St. Andrew, Jamaica, 23 May 1949, in hole in root of tree near cave.

Allotype, male, slide No. 1500, Tom Cringle's tree, near Ferry River, Kingston, Jamaica, 21 May 1949, in deep buttresses of very large Ceiba tree.

Paratypes (mounted), 2 males, 2 females, slides 1429–1432, Springvale, St. Catherine, 21 May, in small cave under large boulder; 1 male, slide 1434, Springvale, St. James, 26 May, 700 ft., in buttress of Ceiba tree; 1 female, slide 1433, Schaw Castle, nr. Springvale, St. James, 26 May, 1250 ft., in buttress of *Ficus* sp. in scrubby forest; 1 male, slide 1481, Rockfort, nr. Kingston, St. Andrew, 20 May, in hole under roots of Ceiba tree; 1 male, 1 female, slides 1501, 1502, Montego Bay, St. James, 1 mi. out on Irwin road, 26 May, in buttresses of Ceiba tree; (unmounted, but examined in phenol and dried on strips of paper in vials) 2 males, same data as slide 1429; 2 males, Stony Hill, St. Andrew, 22 May, 1300 ft., in buttresses of Ceiba tree; 1 male, nr. Albion, St. Thomas, 24 May, in crevices in trunk of large *Ficus* tree; 26 males, 23 females, Melrose Hill, nr. Williams Field, Manchester, 1200 ft., 25 May, in hollows in and under roots of small tree and in buttresses of Ceiba tree; 15 males, 2 females, same data as slide 1501; 8 males, 3 females, Dee Side, Trelawney, 26 May, 350 ft., in buttresses of Ceiba tree.

This species is impossible to distinguish from the other Jamaican form without clearing and microscopic examination. The relatively longer third antennal segment is the only external character we have found to differentiate the two. The pharynx, is, however, quite unique among American *Phlebotomus*, being closely similar to such species as *P. theodori* of Palestine which Theodor (1948) places in the genus *Sergentomyia*. The male genitalia differ from *cayennensis jamaicensis* in having the genital pump smaller and the filaments shorter and considerably more



FIGS. 1–4. Female cibaria of: 1, *P. cayennensis hispaniolae* n. subsp. (Dominican Republic); 2, *P. duppyorum* n. sp. (Jamaica); 3, *P. christophei* n. sp. (Haiti); 4, *P. cubensis* n. sp. (Cuba). FIGS. 5–8. Spermathecae of: 5, *P. cayennensis jamaicensis* n. subsp. (Jamaica; 6, *P. cayennensis hispaniolae* n. subsp. (Dominican Republic); 7, *P. christophei* (Haiti); 8, *P. duppyorum* n. sp. (Jamaica). FIG. 9. Pharynx of *P. duppyorum*. Figs. 1–4 are to the scale indicated to the right of fig. 3; figs. 5–8 by the scale below fig. 8. The figures of spermathecae were drawn from specimens cleared in phenol with the exception of fig. 5 which was mounted in balsam before drawing.

slender. The male pharynx of ***duppyorum*** is more slender and the cibarium shows but faint vestiges of teeth, while the cibarium of ***cayennensis jamaicensis*** shows well marked teeth. The somewhat barbarous name was chosen to commemorate the species' marked preference for Ceiba trees, believed in Jamaica to be also the favorite domicile of "duppies," or ghosts.

Phlebotomus cubensis sp. nov.

Plate I, figs. 3 and 9; Plate II, fig. 4

Male.—A small brownish sandfly with brown mesonotum and dusky wings. Abdominal hairs mixed erect and semi-recumbent. Eyes of moderate size. Third antennal segment slightly shorter than first three palpal segments. Ascoids simple, short, hardly one-fourth the length of their respective segments. Newstead's scales slender, clubbed, in a rather dense patch on the proximal third of the segment. Genitalia as in *cayennensis*, but the genital pump and filaments smaller and shorter than in any of the forms of that species. Cibarium with apparently six or seven short teeth. Pharynx with very minute teeth in groups.

Female.—Externally like the male, but larger. Spermathecae as in *cayennensis*. Cibarium with five teeth in a rudimentary comb, the middle tooth shorter than the others, and with vestiges of another pair of teeth laterally. Pharynx densely spinose, as in *cayennensis*.

Holotype, female, slide no. 1466, Finca Chiquito Ingenio, about 10 mi. East of Camaguey, Cuba, 30 May, 1949, in shallow buttresses of trees in orchard.

Allotype, male, slide 1464, same data as holotype.

Paratypes (mounted), 2 males, 1 female, slides 1465, 1467, 1468, same data as holotype; 1 female, slide 1436, Siete Palmas, 5 mi. South of Camaguey on Carretera Vertientes, 30 May, in crevices in trunk of *Ficus* tree in pasture; 1 male, slide 1437, San Vicente, Vinales valley, 1000 ft., Pinar del Rio, 3 June, in buttress of small tree in dense thickets along stream; (unmounted but examined in phenol and dried on strips of paper in vials) 127 males and females, same data as holotype; 6 males, 2 females, same locality, but from hollow dead tree; 2 males, 1 female, Finca el Milagro, between Minas and Altagracia on Nuevitas road north of Camaguey, 30 May, in buttresses and hollow trees in cut-over forest; 2 males, 1 female, Chapaste, Havana, Prov., 2 June, in crevices in trunk of *Ficus* tree; 14 males, 4 females, 7 km. west of Ceiba Mocha, Matanzas Prov., 2 June, in buttresses of large Ceiba trees in scrubby forest; 6 males, 2 females, same locality as slide 1437 but 4 June.

Phlebotomus cayennensis jamaicensis, subsp. nov.

Plate I, fig. 11; Plate II, fig. 5

Male.—A small, somewhat dusky sandfly, the degree of infuscation of wings and mesonotum varying somewhat. Third antennal segment slightly shorter than first three palpal segments. Newstead's scales few, in a loose patch near the proximal third of the segment. Ascoids simple, short, about one-fifth length of their respective segments. Genitalia as in other races of *cayennensis*, but the pump and genital

filaments heavier and longer than in any of the others, being most closely approached by *viequesensis* in this respect. Cibarium with a well marked comb of short but distinct teeth, 12 to 15 in number. Pharynx unarmed.

Female.—Like the male externally and in color, third antennal segment relatively shorter than in male. Newstead's scales more numerous and in a denser patch. Spermathecae somewhat collapsed in the single available specimen, rather large, equalling or exceeding those of *viequesensis* in size. Cibarium with a comb of 13 to 15 long horizontal teeth and apparently four erect teeth in the middle. This structure differs from the cibarium of *viequesensis* apparently only in having the teeth rather heavier and longer. Pharynx rather heavily sclerotized, densely spinose at apex.

Holotype, female, slide 1480, Rockfort, nr. Kingston, St. Andrew, Jamaica, 20 May 1949; in hole under Ceiba tree.

Allotype male, slide 1479, same data as holotype.

Paratypes, 1 male, slide 1435, same locality and date as holotype, but in a shallow cave in side of quarry a few yards from Ceiba tree; 1 male, slide 1503, Ferry River, nr. Kingston, St. Andrew, Jamaica, 23 May, 1949, in crevices in rock near entrance to a large shallow cave.

This form is exceedingly close to *cayennensis viequesensis*, but appears to be larger. Additional material will be needed to be certain that the small differences noted are really significant. It appears to be rare in Jamaica, where it was found only in the immediate vicinity of Kingston, and may represent a rather recent importation, perhaps from one of the lesser Antilles.

Phlebotomus cayennensis hispaniolae subsp. nov.

Plate I, fig. 8; Plate II, figs. 1 and 6

Male.—A small, rather dark, sandfly with dusky wings. The mesonotum is quite blackish, contrasting with the paler pleura. Abdomen dusky, as are the tibiae, abdominal hairs mostly erect or semi-erect. Third antennal segment a little shorter than first three palpal segments. Newstead's scales few, in a small patch on the proximal third of the segment. Acoids simple, short, about one-fifth of their respective segments. Cibarium with a comb of 8 to 10 teeth, unusually long and well developed for a male. Pharynx apparently unarmed. Genitalia as in other races of the species, the pump and filaments indistinguishable from those of *cayennensis puertoricensis*.

Female.—Coloration, etc., as in the male, but about one-third larger throughout. Cibarium with 12 to 15 teeth, like *puertoricensis*, but the teeth apparently somewhat shorter. Pharynx spinose proximally. Spermathecae with moderate sized heads but unusually thick and short ducts, much shorter and thicker than those of *puertoricensis*.

Holotype, female, slide 1478, 16 km. South of Hato Mayor, Prov. Seibo, Dominican Republic, 12 June 1949, in buttresses of large Ceiba tree.

Allotype, male, slide 1472, 34 km. South of Sabana de la Mar, Prov. Seibo, Dominican Republic, 12 June, 1949, in buttresses of large tree in coffee plantation.

Paratypes (mounted) 1 male, 2 females, same data as allotype, slides 1473, 1470, 1471; 1 female, same data as holotype, slide 1477; 1 female, 13 km. N. W. of Ciudad Trujillo, Dominican Republic, 11 June 1949, in small hollow tree in pasture, slide 1441; 1 male, Milot, nr. Cap Haitien, Haiti, 9 June 1949, in hole in ruins, slide 1439; (unmounted, but examined in phenol and placed on slips of paper in vials) 8 males, 21 females, 19 km. West of Port au Prince on Leogane road, Haiti, 7 June 1949, in buttresses of large Ceiba trees; 12 females, 19 males, Charrier, 2 km. from Cap Haitien on Limbe road, Haiti, 8 June 1949, in buttresses and holes in logwood trees in small patch of forest; 6 males, 3 females, La Voute, 4.8 miles from Cap Haitien on Limbe road, Haiti, 9 June 1949, in buttresses of door yard trees; 3 males, 2 females, Cap Haitien, Haiti, 9 June 1949, in holes in ruins of Pauline Bonaparte's palace on point East of town; 5 males, 7 females, Milot, nr. Cap Haitien, Haiti, 9 June 1949, 400 ft., in holes in ruins of palace of Sans Souci; 1 male, 2 females, 15 km. South of Cap Haitien on Milot road Haiti, 9 June 1949, in large hollow Calabash tree; 10 males, 1 female, 16 km. South of Hato Mayor, Prov. Seibo, Dominican republic, 12 June 1949, in buttresses of Ceiba tree; 14 males, 8 females, 34 km. South of Sabana de la Mar, Prov. Seibo, Dominican Republic, 650 ft., 12 June 1949, in buttresses and under loose bark of large trees in coffee plantation; 1 female, 6 km. South of Sabana de la Mar, Prov. Seibo, Dominican Republic, 12 June 1949, in buttresses of large trees in Cacao plantation; 2 males, 2 females, Rincon, between Monsenor Nouel and La Vega, Prov. de la Vega, Dominican Republic, 13 June 1949, in hollow mango tree in old cacao plantation, 500 ft.; 2 males, Rio Verde, 12 km. South of Moca, Prov. Espaillat, Dominican Republic, 14 June 1949, in buttresses of large Ceiba tree on river bank; 4 males, 2 females, nr. Colonia Pedro Garcia, between Santiago and Puerto Plata, Prov. Puerto Plata, Dominican Republic, 14 June 1949, 1900 ft., between roots of large *Cecropia* tree in secondary forest.

This race differs from the Puerto Rican form mainly in the structure of the spermathecae, the minor differences in cibarium, etc., being possibly not significant.

Phlebotomus cayennensis puertoricensis Fairchild and Hertig

1948. Ann. Ent. Soc. Amer., 41: 462.

In addition to the material previously reported (1948), we now have specimens from the following localities in Puerto Rico, collected by the junior author.

Eleven males, 2 females, Toro Negro Unit of Caribbean National Forest, near Kilometre post 47 on Route 11, northwest of Villalba, 2200 ft., 23 Sept. 1948, taken from holes and crevices among roots in roadside bank. (This is the highest elevation at which *Phlebotomus* were taken in Puerto Rico, though searched for up to 3200 ft. in the Luquillo Unit of the National Forest); 3 females, near Kilometre post 67 on Route 1, between Cayey and Aibonito, 1000 ft., 21 Sept. 1948; 2 males, 5.8 miles south of Canovanas on Route 43, 650 ft., 25 Sept. 1948, taken from a large crevice between boulders near a small stream.

Phlebotomus cayennensis viequesensis Fairchild and Hertig

1948. Ann. Ent. Soc. Amer., 41: 464.

As previously noted, the junior author took this form in some numbers on St. Thomas in the Virgin Islands. All the material was taken from drainage holes in stone retaining walls in and near the town of Charlotte Amalie between 18 and 19 Sept. 1948. Collecting was difficult, as the constant strong trade winds blew the sandflies away as soon as they were driven to the entrances of the holes by tobacco smoke.

Seven males, 6 females, Denmark Hill, 19 Sept.; 1 male, Catherine Berg 88, 19 Sept.; 7 males, 9 females, Queen's Quarters, Bjerge Gade, 18 Sept.

Phlebotomus atroclavatus Knab

1913. Ins. Ins. Mens 1: 135; Fairchild and Hertig, 1948, Ann. Ent. Soc. Amer., 41: 455.

Through the kindness of Dr. Harry D. Pratt, of the U. S. Public Health Service, we have been enabled to examine two males of this species taken by Kohler near Mt. Washington, N. W. St. Croix, Virgin Islands, 21 Sept. 1949. This extends considerably the range of this species in the West Indies, as it was hitherto known with certainty only from Guadeloupe in the French West Indies.

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Fairchild, G. B., and **Marshall Hertig.** 1948. Notes on the Phlebotomus of Panama. IV. *P. atroclavatus*, *P. cayennensis*, *P. chiapanensis* and some related forms from the West Indies and Mexico. Ann. Ent. Soc. Amer., 41 (4): 462–466.

Theodor, O. 1948. Classification of the Old World species of the subfamily *Phlebotominae*. Bull. Ent. Res., 39 (1): 110, Pl. XI, fig. 2.

UNDESCRIBED SPECIES OF JAPANESE CRANE-FLIES

(Diptera: Tipulidae)

PART VII

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The initial part of this series of papers was published in the Annals of the Entomological Society of America, 12 : 327–348, 1919; the preceding part in the Annals, 40 : 350–371, 1947. In the present report I am construing the limits of the paper as including the former Japanese Empire and am discussing species not only from the main islands of Japan but also from Manchuria, Korea and Formosa. Except where indicated to the contrary the types of the new species are preserved in my personal collection of crane-flies.

For a discussion of conditions in northern Korea where very extensive series of crane-flies were taken by Mr. Alexander M. Yankovsky between 1937 and 1940, a paper by the writer should be consulted.[1] For the itinerary of the Japanese expedition to Manchuria, including Jehol, the report by Tokunaga[2] may be consulted.

Genus **Tipula** Linnaeus

Tipula (Yamatotipula) nigrolamina n. sp.

General coloration gray, the praescutum with four inconspicuous darker gray stripes that are faintly margined with darker; nasus lacking; flagellar segments incised; wings with a brownish yellow tinge, only slightly variegated; stigma oval, brown; *Rs* about two and one-half times *m-cu*; male hypopygium with the tergite transverse, the caudal margin produced into two conspicuous obtuse lobes that are provided with black setae; gonapophyses with unusually short stems, the large blades blackened.

Male.—Length, about 15 mm.; wing, 17.5 mm.; antenna, about 4.1 mm.

Frontal prolongation of head relatively long, subequal to remainder of head, black, heavily pruinose, especially on dorsum; nasus lacking; palpi black. Antennae with scape brownish black, pruinose; pedicel dark brown, flagellum black; flagellar segments relatively short, conspicuously incised; longest verticils about as long as the segments; terminal segment nearly one-half as long as the penultimate, slender.

[1]Alexander, Charles P. 1945. Undescribed species of crane-flies from northern Korea (Diptera, Tipuloidea). Trans. R. Ent. Soc. London, 95: 227–246.

[2]Tokunaga, Shigeyasu. 1934. Natural science research of the first scientific expedition to Manchoukuo. Sect. 1: 1–76, 391 figs., map; itinerary in English, pp. 45–76.

Head light gray; vertical tubercle developed as a tiny conical protuberance; anterior vertex broad, nearly five times the diameter of scape, the eyes correspondingly small.

Pronotal scutum gray, weakly infuscated medially; scutellum and pretergites yellow. Mesonotal praescutum light gray, with four darker gray stripes that are very inconspicuous against the ground; a capillary dark brown median vitta, the remaining darkened borders less conspicuous but evident; praescutal interspaces very inconspicuous; scutum gray, the lobes with scarcely darkened rings; scutellum gray, variegated with three pale lines, including a median dash; mediotergite clearer gray; pleurotergite gray, the katapleurotergite more yellowed. Pleura gray, the dorsopleural membrane broadly yellow. Halteres with stem obscure yellow, base of knob weakly darkened, the apex again pale. Legs with the coxae light gray; trochanters yellow; femora obscure yellow, the tips very gradually more blackened; tibiae brownish black; tarsi black; claws long, with conspicuous tooth. Wings with a brownish yellow tinge, the cells beyond cord a trifle more darkened; prearcular field and cell *Sc* clearer yellow; stigma oval, brown, its proximal portion somewhat more yellowed; cells beyond stigma a trifle more whitened; obliterative area across cell *1st* M_2 poorly indicated; veins brown, yellow in the flavous fields. Macrotrichia of veins beyond cord conspicuous and relatively abundant. Venation: *Rs* long and nearly straight, about two and one-half times the oblique *m-cu*; R_{1+2} entire; petiole of cell M_1 subequal to *m*; *m-cu* on M_4 shortly beyond origin of latter.

Abdomen with first tergite dark gray, bordered by yellow; succeeding tergites reddish, with indications of two broken stripes, these very faint and poorly indicated on the more proximal segments, becoming distinct on about the fifth segment; lateral borders of tergites broadly, the caudal margins more narrowly yellow; basal sternites yellow, the outer segments darker and more pruinose; outer segments, including hypopygium, conspicuously blackened. Male hypopygium with the tergite transverse, the caudal margin produced into two conspicuous obtuse lobes that are separated by a smaller rounded notch; vestiture of lobes appearing as abundant long black setae but without spines. Basistyle rather strongly produced caudad so that its apex lies almost as far distad as does the apex of the ninth tergite. Outer dististyle arcuated, dusky, gradually narrowed to the slender obtuse tip. Inner dististyle without conspicuous spines, as in *ompoensis* and some other species, generally triangular in outline, the rostral portion narrowed, its ventral margin produced into a low lobe or flange, with a small blackened point immediately beneath it; no developed outer basal lobe, its position heavily blackened and thickened but not produced. Ninth sternite with the median region broadly membranous behind and bearing a transverse flattened hairy lobe. Gonapophysis appearing as an unusually large blackened blade, more or less mitten-shaped, with the basal stem unusually short. Eighth sternite unarmed.

Habitat: Northern Korea. *Holotype*, ♂, Seren Mountains, altitude 3000 feet, June 26, 1938 (Yankovsky).

Other generally similar species in Japan and Korea include *Tipula* (*Yamatotipula*) *kamikochiensis* Alexander, *T.* (*Y.*) *machidai* Alexander, *T.* (*Y.*) *ompoensis* Alexander, and *T.* (*Y.*) *sempiterna* Alexander. The

present fly differs very conspicuously in the structure of the male hypopygium, especially the tergite, inner dististyle and gonapophysis.

Tipula (Arctotipula) conjuncta conjunctoides n. subsp.

Male.—Length, about 20 mm.; wing, 19 mm.; antenna, about 6 mm.

Described from an alcoholic specimen.

Characters as in typical *conjuncta*, differing as follows:

Antennae weakly bicolored, the extreme proximal end of each segment obscure yellow, the remainder dark; distal end of individual flagellar segments not produced, the outer end being narrower than the basal enlargement (compare typical *conjuncta* Alexander, 1925 Encycl. Entomol., Diptera, 2 : 88–89, fig. 2, antenna, and fig. 3, venation).

General coloration of thorax (in alcohol) dark gray, the abdomen chiefly obscure yellow. Legs longer and more slender than in typical *conjuncta*, this probably a sexual character. Wing pattern almost exactly as in *conjuncta*, the veins a little darker. Macrotrichia of veins lacking or virtually so on *Rs* and its anterior branch, abundant on distal end of vein R_{4+5} and on distal ends of outer medial branches. Venation: Cell R_3 with inner end more pointed, cell R_2 at margin being correspondingly widened, about one-half as extensive as cell R_3; inner end of cell *1st* M_2 less pointed; cell M_1 much deeper, approximately three times its petiole.

Male hypopygium large and powerfully constructed. Ninth tergite extensive, depressed, the caudal margin with a deep, parallel-sided median notch, the very broad lateral lobes truncated at apex, the outer lateral portion rounded; dorsal surface of tergite with abundant short dense setae. Inner dististyle produced into a very long slender simple beak; entire outer surface of style with coarse setae. Eighth sternite unarmed, without lobes or hair-pencils.

Habitat: Manchuria (Jehol).

Holotype, alcoholic ♂, No. 25, without more specific data. See reference to the Manchoukuo Expedition in introduction to this paper.

Typical *conjuncta* is still known only from the Ussuri District, eastern Siberia. In its general appearance the present fly is very similar and I would have considered the two as being identical if it were not for the structure of the antennae and the venation. In size, and in coloration of the body and wings, the two flies are very similar to one another. They may be separated by the following key:

1. Flagellar segments with outer end slightly produced to form a weak serration; cell M_1 of wings shallow, shorter than its petiole. . . . . **conjuncta conjuncta**
   Flagellar segments widest at the basal enlargement, the distal end narrow; cell M_1 deep, approximately three times its petiole. . **conjuncta conjunctoides**

The subgeneric position of *conjuncta* had been held in question (Alexander, Philippine Journ. Sci., 1935, 57 : 85) but its reference to *Arctotipula* Alexander now seems fully justified since the general structure of the male hypopygium is very suggestive of that of *Tipula* (*Arctotipula*) *miyadii* Alexander, of the Kurile Islands. The species is told from all other now rather numerous members of the subgenus by the fusion of veins R_{4+5} and M_{1+2}, obliterating the *r-m* crossvein.

Tipula (Arctotipula) laterodentata n. sp.

General coloration gray, the praescutum with four darker stripes that are incompletely bordered with darker brown; nasus lacking; antennae black, the more basal flagellar segments with their proximal portions brightened; femora brownish yellow, the tips blackened; claws (male) toothed; wings brownish yellow, restrictedly patterned with darker, including the blackened stigma; basal abdominal tergites reddish, with a broad black central stripe, the first tergite, sternite and outer segments more uniformly dark gray; ninth segment blackened; male hypopygium with outer angle of tergal lobe produced into an acute spine; outer dististyle conspicuously dilated at near midlength; inner dististyle at apex expanded into a blade, the rostral portion glabrous, the upper angle with powerful setae; face of style with a strong blackened spine.

Male.—Length, about 16–17 mm.; wing, 16.5–18 mm.; antenna, about 3.3–3.6 mm.

Frontal prolongation of head moderately long, dark gray, without nasus; palpi black, relatively short, especially the terminal segment. Antennae relatively short, black, the scape and pedicel gray pruinose; bases of proximal four or five flagellar segments restrictedly obscure yellow, the remainder black; flagellar segments subcylindrical, with scarcely indicated basal enlargements, the verticils exceeding the segments. Head gray, the vertex with a broad brown central stripe; eyes relatively small, anterior vertex unusually broad, nearly five times the diameter of scape.

Pronotum gray, somewhat weakly infuscated medially, the scutellum and pretergites obscure yellow. Mesonotum gray, the praescutum with four darker gray stripes, the outer borders of the intermediate pair and the inner margins of the lateral stripes darker brown; each scutal lobe with two brownish gray areas; mediotergite with a central infuscation or short stripe; vestiture of mesonotum relatively long but sparse, almost white. Pleura gray, the dorsopleural membrane buffy yellow, conspicuously variegated on dorsal portion with dark brown. Halteres with stem yellow, knob brownish black. Legs with coxae light gray; trochanters somewhat darker gray; femora brownish yellow, the tips blackened, the amount subequal on all legs but with the posterior pair more fulvous yellow, preceding the darkened portions; tibiae black, the bases restrictedly obscure yellow; tarsi black; claws (male) with strong subbasal tooth. Wings with the ground brownish yellow, restrictedly patterned with darker; prearcular field extensively yellowed; stigma oval, conspicuously blackened; cell *Sc* weakly infuscated; cord and most of the veins beyond it insensibly seamed with darker, most evident over the posterior cord and distal ends of the outer radial cells; veins dark brown, yellow in the prearcular field. No squamal setae; veins unusually glabrous, as is common in the subgenus, beyond the cord with an extensive series over most of the outer section of R_{4+5}. Venation: R_{1+2} entire but short and oblique; *Rs* relatively long, about two and one-half times *m-cu;* petiole of cell M_1 subequal to *m;* cell *1st* M_2 relatively long and narrow, its inner end more or less pointed.

Basal abdominal tergite gray, the succeeding four tergites conspicuously reddened, with a broad black central stripe that is narrowly

interrupted at the posterior borders of the segments; lateral tergal borders light gray, vaguely bordered internally by more brownish gray; sternites and outer tergites gray, more or less patterned with more reddish areas; ninth segment, excepting the outer dististyle, blackened. Male hypopygium with the lateral lobes of tergite broad, truncated, separated by a deep U-shaped median notch; outer angles of lobes produced into strong chitinized points; ventral surface of mesal angles with a strong blackened chitinized blade or flange; tergite with abundant black setae, on the mesal portions even more dense and conspicuous. Outer dististyle widely dilated at near midlength, its greatest width exceeding one-half the length, the more narrowed apex obtuse. Inner dististyle moderately wide, at apex dilated into a truncated blade, the lower or rostral portion subglabrous, the upper part with about eight or nine powerful setae; face of style with a strong blackened spine.

Habitat: Northern Korea. *Holotype*, ♂, Seren Mountains, altitude 2500 feet, June 15, 1938 (Yankovsky). *Paratopotypes*, 6 ♂♂.

Readily told from the other regional members of the subgenus by the coloration of the body and antennae and especially by the structure of the male hypopygium, particularly the tergite and inner dististyle. The characters of the subgenus and a list of the regional species have been given by the writer in earlier papers (1933, Philippine Journ. Sci., 52 : 410–411; 1935, *idem*, 57 : 116–117). Lackschewitz (1936, Trav. Inst. Zool. Acad. Sci. URSS, 4 : 245–312) has attempted to synonymize certain of the Nearctic species of *Arctotipula* described by the writer with other common and widespread Arctic forms, specifically *Tipula* (*Arctotipula*) *aleutica* Alexander with *T.* (*A.*) *besselsi* Osten Sacken, the latter the subgenotype, and *T.* (*A.*) *alascaensis* Alexander with *T.* (*A.*) *ciliata* Lundstrom of Arctic Eurasia. I can affirm that in neither of these instances is the placing in synonymy justified and both species are entirely valid. Actually *besselsi* is very close to the commonest species in Arctic Europe, *T.* (*A.*) *salicetorum* Siebke (*nigricornis* Zetterstedt, not Macquart), both having a peculiar blackened sclerotized armature on the ventral surface of the ninth tergite. It is becoming increasingly evident that there are relatively numerous species in the Holarctic Region, some closely interrelated yet nevertheless quite distinct. Attention again may be called to the fact that the northern European *T.* (*A.*) *tumidecornis* Lundstrom and the Nearctic *T.* (*A.*) *illustris* Doane (*fuscipennis* Loew, 1865, nec Curtis, 1834) are actually species of *Arctotipula* and not members of the genus *Prionocera* Loew, where they have commonly been placed.

Tipula (Arctotipula) mediodentata n. sp.

Size large (wing, 18 mm.); general coloration gray, the praescutum with four darker brownish gray stripes; nasus elongate; antennae with pedicel obscure yellow, flagellum brownish black; femora yellow, the tips conspicuously blackened; claws (male) toothed; wings brownish yellow, weakly patterned with darker; stigma oval, dark brown, in male with about 25 trichia, glabrous in female; veins beyond cord with numerous trichia; male hypopygium with the tergite narrowed outwardly, the caudal margin with a broad but deep U-shaped median notch, the sides of lobes adjoining the notch produced caudad into

slender setuliferous points; outer dististyle greatly expanded outwardly; inner dististyle with the outer basal lobe a blackened spine, with two further smaller spines on margin; gonapophysis with abundant microscopic tubercles.

Male.—Length, about 16 mm.; wing, 18 mm.; antenna, about 4 mm.

Female.—Length, about 17 mm.; wing, 18 mm.

Frontal prolongation of head relatively short, dark gray; nasus conspicuous, nearly one-half the length of the prolongation, truncated at apex; palpi with basal three segments dark brown, the terminal one pale yellowish brown. Antennae with scape light brown, gray pruinose, pedicel obscure yellow, flagellum brownish black; flagellar segments (male) relatively short, moderately incised, with the basal enlargements becoming well-indicated on the more distal segments; terminal segment more than three-fourths the length of the penultimate; longest verticils slightly exceeding the segments in length. Head light gray, a trifle more infuscated on the broad vertex, particularly in female; vertical tubercle not or scarcely indicated; vestiture of vertex relatively long, blackened.

Pronotum infuscated medially, gray on sides; scutellum and pretergites light yellow. Mesonotum gray, the praescutum with four darker brownish gray stripes, much more distinct and contrasted in female, the stripes entire or with the mesal edges of the intermediate pair slightly more darkened; scutal lobes conspicuously patterned with brownish gray; scutellum broadly dark brown; mediotergite with a much narrower capillary brown central stripe; pleurotergite with the katapleurotergite more infuscated. Pleura gray, somewhat darker on the anepisternum, the dorsopleural membrane broadly light yellow; in female, the membrane more infuscated, especially posteriorly. Halteres obscure yellow, the base of knob restrictedly more darkened. Legs with the coxae gray, with long setae; trochanters yellow; femora yellow, the tips conspicuously blackened, the amount approximately equal on all legs; tibiae and basitarsi light brown, darker at tips, remainder of tarsi black; claws (male) toothed. Wings with a weak brownish yellow tinge, darker beyond cord; prearcular field and cell Sc more yellowed; stigma oval, dark brown, conspicuous, provided with about 25 trichia in male, glabrous in female; narrow pale brown seams on cord and outer end of vein M_3; veins beyond cord and the axillary border less evidently seamed with brown. Squama naked; veins beyond cord with abundant trichia, particularly in radial field, becoming more sparse in the medial field, on M_4 only at base. Venation: *Rs* about two and one-half times *m-cu*; R_{1+2} relatively short, pale and with trichia only near origin; in female, vein R_3 longer and more extended; second section of M_{1+2} arcuate, widening cell *1st* M_2; m about twice the petiole of cell M_1.

Abdomen of male with basal tergite gray, with a brown central spot; succeeding tergites yellow with a broad brownish black median stripe that is broadly interrupted by the yellow posterior borders of the segments, lateral tergal borders paling to gray; sternites gray, the caudal borders narrowly yellow; outer segments, including hypopygium, more uniformly blackened, the outer dististyle conspicuously yellow. In the female, the abdomen is somewhat the same but the gray lateral tergal borders are margined internally by somewhat paler brown, not forming

a distinct sublateral stripe; pleural membrane dark. Ovipositor with the genital shield and the preceding segment dark brown; cerci compressed, yellow, the margins smooth; hypovalvae much deeper, their tips truncated. Male hypopygium with the tergite large, the basal third glabrous, the outer portion with dense black setae; outer and narrowed to give a general convex appearance to the distal portion, the median region with a deep and broad U-shaped notch; sides of notch produced caudad into slender yellow points that are densely setuliferous. Outer dististyle greatly expanded at outer end, appearing generally triangular in outline, the width across the truncated apex exceeding two-thirds the length of the style. Inner dististyle elongate, strongly bent at near midlength; beak more or less truncate, its sides with a conspicuous blackened lateral flange back from the apex; outer basal lobe a short but powerful blackened spine; margin of style basad of this spine with two smaller erect spinous points. Gonapophysis widened outwardly, the tip membranous, the surface with abundant microscopic tubercles.

Habitat: Northern Korea. *Holotype*, ♂, Chonsani, altitude 4300 feet, July 14, 1937 (Yankovsky). *Allotopotype*, ♀, pinned with type. *Paratopotype*, ♀, same data.

Although generally similar to *Tipula* (*Arctotipula*) *laterodentata* n. sp., the present fly is entirely distinct, particularly in the nasus and in the structure of the male hypopygium, as the tergite, both dististyles and the gonapophysis.

Tipula (Acutipula) bicompressa n. sp.

Allied to *bipenicillata;* size large (wing, male, over 20 mm.); wings yellowish brown, the prearcular and costal fields more yellowed; obliterative band conspicuous; male hypopygium with the median lobe of ninth tergite unusually slender; inner dististyle with the outer beak produced into a bulbous or spatulalike lobe, with a small slender spine near its base; eighth sternite sheathing, on either side of apical portion with a compressed flattened lobe that is densely covered with short setae.

Male.—Length, about 20 mm.; wing, 21 mm.; antenna, about 5.1 mm.

Frontal prolongation of head blackened, sparsely pruinose above at base; nasus elongate; palpi black, incisures restrictedly brightened; terminal segment not exceeding twice the penultimate. Antennae of moderate length; scape and pedicel yellow; first flagellar segment obscure yellow, restrictedly darkened at base; succeeding segments very vaguely bicolored, darkened at base, the stems a trifle paler; outer segments more uniformly dark brown. Head gray; eyes large; anterior vertex relatively narrow, only about one and one-half times the greatest diameter of scape.

Pronotum obscure yellow. Mesonotal praescutum and scutum more brownish gray, the former without clearly defined stripes; scutellum and postnotum more yellow pollinose. Pleura, including dorsopleural membrane, uniformly yellow. Halteres with stem yellow, knob infuscated. Legs with the coxae and trochanters yellow; remainder of legs elongate; femora and tibiae obscure yellow; the tips very narrowly and inconspicuously darkened; tarsi brown, the outer segments passing into black; claws (male) toothed. Wings with a strong yellowish brown

tinge, the prearcular and costal fields clearer yellow; stigma oval, darker brown; obliterative band along cord conspicuous, entering the base of cell M_3; veins dark brown, more yellowed in the brightened fields. Squama with trichia; veins beyond cord without trichia, excepting a restricted series along vein R_{4+5}. Venation: R_{1+2} entire; cell R_2 narrowed at base; *m* a little exceeding the petiole of cell M_1; *m-cu* a short distance before the fork of M_{3+4}.

Abdomen with basal tergites obscure yellow, the outer ones somewhat more darkened medially; caudal borders of tergites very narrowly, the lateral margins more broadly pale yellow; basal sternites yellow; outer segments passing into black. Male hypopygium with the ninth tergite produced into an unusually slender lobe, the tip entire, densely set with short blackened spines. Outer dististyle relatively small, widest at near midlength, the tip obtuse. Inner dististyle small; beak small and slender, outer beak produced into a bulbous or spatulate lobe, its lower margin further produced into an obtuse flange; between the two beaks with a small straight spine; outer margin of style with abundant erect setae. Eighth sternite sheathing, on either side of the apical portion with a conspicuous longitudinal compressed-flattened lobe that is densely covered with short setae; median space between lobes wide, filled with pale membrane.

Habitat: Northern Korea. *Holotype*, ♂, Puksu Pyaksan, altitude 2500 feet, June 8, 1939 (Yankovsky).

Tipula (*Acutipula*) *bicompressa* is most similar to *T.* (*A.*) *bipenicillata* Alexander and *T.* (*A.*) *tokionis* Alexander, differing from both particularly in the structure of the male hypopygium, including the tergite, both dististyles, and the eighth sternite.

Tipula (Lunatipula) sublimitata atrodeclivis n. subsp.

Male.—Length, about 19 mm.; wing, 19 mm.; antenna, about 6.3 mm.

Generally similar to typical *sublimitata* Alexander, of Kamtchatka, differing chiefly in the structure of the male hypopygium, as follows: Ninth tergite with the mesal faces of the tergal lobes heavily blackened and precipitous. Outer dististyle dilated into a slightly flattened blade. Inner dististyle with beak very slender; outer basal lobe a small thumblike lobe. Eighth sternite with the lateral lobes relatively short and stout, blackened, each lobe narrowed outwardly, terminating in an acute sclerotized point, the face and mesal margin with abundant long coarse setae; beneath the lateral lobes and closer to midline with a pair of smaller darkened obtusely pointed lobes. In the typical form, the lateral lobes of the eighth sternite are elongate, decussate across the midline, their tips expanded, blunt.

Habitat: Northern Korea. *Holotype*, ♂, Seren Mountains, altitude 5000 feet, August 2, 1938 (Yankovsky).

Genus **Dicranota** Zetterstedt

Dicranota (Dicranota) profunda n. sp.

General coloration of thorax brownish yellow, the pleura and posterior sclerites clearer yellow; legs brownish yellow; wings uniformly

pale yellow; R_{2+3+4} long, subequal to or exceeding *m-cu*; cell M_2 open; abdomen brownish yellow, the subterminal segments darkened to form a ring; male hypopygium with the tergite profoundly incised medially, the notch unusually deep and narrow, each lateral lobe further subdivided by a smaller emargination, the intermediate lobules thus formed long and slender.

Male.—Length, about 5–5.5 mm.; wing, 5.5–6 mm.

Female.—Length, about 5.5–6 mm.; wing, 6.3–7 mm.

Rostrum brownish yellow; palpi pale brown. Antennae relatively short; scape yellow, remainder of organ black. Head dark gray, paler behind.

Thorax obscure yellow or brownish yellow, the pleura and posterior sclerites of notum clearer yellow; in cases, the pronotum and praescutum a trifle darker. Halteres with stem yellow, knob weakly infuscated. Legs with the coxae and trochanters yellow; remainder of legs obscure brownish yellow, the outer segments passing into pale brown. Wings uniformly pale yellow; stigma barely indicated; veins pale brown, a little more yellowed in the basal and costal regions. Venation: *Sc* moderately long, Sc_1 ending about opposite the fork of R_{2+3+4}, Sc_2 shortly before midlength of the distance between arculus and origin of *Rs;* supernumerary crossvein in cell R_1 usually present, in cases very faint to lacking; R_{2+3+4} long, subequal to or exceeding *m-cu*; R_2 far distad, nearly perpendicular, longer than R_{1+2}; basal section of R_5 very reduced to lacking, *Rs* being in virtual alignment with the second section of the vein; cell M_2 open; *m-cu* opposite or before midlength of M_{3+4}.

Abdomen with tergites brownish yellow, the posterior borders clearer yellow; sternites clear yellow; subterminal segments more darkened to form a ring; styli of hypopygium obscure yellow. Male hypopygium with the tergite profoundly incised medially, the notch unusually deep and narrow, each lobe further subdivided by a smaller emargination, the intermediate lobules thus formed long and slender, jutting beyond all other parts of the tergite, obtuse at apex and tipped with a few short setae. Basistyle with interbase a broad flattened blade, irregular in outline, widest about opposite midlength, the bulging outer margin with microscopic crenulations or weak serrulations; inner margin of interbase with a short spinous point; extreme apex of basistyle short-pointed, provided with three or four stout spinous setae. Two dististyles, both elongate, the outer cylindrical, with abundant stout setae, those nearest apex stronger and subspinous; inner style about as long, its basal third a little dilated, the outer part a flattened blade with the apex obtusely rounded.

Habitat: Japan (Honshu). *Holotype*, ♂, Funakosi, Iwateken, altitude 100 meters, May 21, 1947 (H. Yamamoto). *Allotopotype*, ♀. *Paratopotypes*, 4 ♂ ♀, pinned with the types.

The most similar regional species is *Dicranota* (*Dicranota*) *nippoalpina* Alexander, of the Japanese Alps, which differs very strikingly in all details of structure of the male hypopygium. The superficially somewhat similar species of the subgenus *Rhaphidolabis*, including *D.* (*R.*) *consors* Alexander and *D.* (*R.*) *subconsors* Alexander, have entirely distinct male genitalia.

Genus **Paradelphomyia** Alexander

Paradelphomyia (Oxyrhiza) nimbicolor n. sp.

Thorax almost uniformly dark brown or brownish black; antennae short, black throughout; wings of male widest opposite the termination of vein *2nd A*, strongly suffused with dusky; male hypopygium with the dististyles terminal in position.

Male.—Length, about 5 mm.; wing, 5.3 mm.

Female.—Length, about 6.5 mm.; wing, 6.5 mm.

Rostrum and palpi black. Antennae short, black throughout; proximal two flagellar segments more or less united into a fusion segment; outer flagellar segments long-cylindrical, with long conspicuous verticils. Head black, sparsely pruinose.

Mesonotal praescutum almost uniformly dark brown or brownish black, the remainder of notum a trifle paler. Pleura brownish black. Halteres with stem weakly infuscated, narrowly paler at base, knob dark brown. Legs with coxae and trochanters testaceous; remainder of legs dark brown, the tarsi passing into black; tibial spurs distinct. Wings of male broadest opposite the termination of vein *2nd A*, strongly suffused with dusky, the stigma a little darker brown; wing base a little more yellowed, including the veins; remaining veins brown. Macrotrichia of outer cells relatively abundant, in male from cell R_1 through cell M_3, in the female even more extensive, into cell M_4. Venation: Sc_1 ending about opposite the fork of Rs, Sc_2 some distance from its tip, Sc_1 alone subequal to R_{2+3+4}; R_2 faintly indicated to lacking, when present placed at or before the fork of R_{3+4}; R_{2+3+4} in virtual longitudinal alignment with vein R_3; veins R_3 and R_4 divergent, cell R_3 at margin being nearly twice cell R_2; cell M_1 present, about one-half its petiole or slightly more; *m-cu* close to midlength of cell *1st* M_2.

Abdomen brownish black, the hypopygium a trifle paler. Male hypopygium with the dististyles terminal in position; outer style ending in two major slightly curved spines, the subterminal spine erect and nearly straight. Phallosomic region injured in the unique type slide; before this damage occurred, an elongate pale slender rod, presumably the aedeagus, was noted as being present.

Habitat: Japan (Honshu). *Holotype:* ♂, Funakosi, Iwateken, altitude 100 meters, September 26, 1947 (H. Yamamoto). *Allotopotype*, ♀, pinned with type. *Paratopotypes*, 2 ♀ ♀.

The present fly is very distinct from the other regional species in the structure of the male hypopygium, especially the terminal dististyles. In the most similar regional species, *Paradelphomyia* (*Oxyrhiza*) *ariana* (Alexander) and *P.* (*O.*) *nipponensis* (Alexander), the apex of the basistyle is produced beyond the point of origin of the dististyles as a strong spine. The European members of the subgenus likewise have the dististyles of the hypopygium terminal in position but differ from the present fly in various other regards, including coloration and venation. In its general appearance, the present fly is somewhat more like *ariana* than it is to other regional species.

Paradelphomyia (Oxyrhiza) chosenica n. sp.

Size large (wing, female, over 7 mm.); general coloration black, the

surface obscured by a gray pruinosity; halteres yellow; femora light yellow, the tips narrowly and vaguely darkened; wings brownish yellow, clearer yellow in the prearcular and costal fields; abundant macrotrichia in outer cells of wing; cell M_1 small; *m-cu* at or before midlength of cell *1st* M_2.

Female.—Length, about 8 mm.; wing, 7.5 mm.

Rostrum and palpi black. Antennae with scape, pedicel and basal segment of flagellum black, the remainder broken. Head black, opaque by a gray pruinosity; anterior vertex broad, exceeding three times the diameter of scape.

Prothorax and mesothorax black, the color obscured by a gray pruinosity, heaviest on the lateral portions of the praescutum, scutellum, postnotum and pleura; anterior lateral pretergites obscure yellow. Halteres yellow. Legs with the fore coxae black; middle coxae blackened, the tips pale; posterior coxae obscure yellow; trochanters yellow; femora light yellow, the tips narrowly and vaguely darkened; base of tibiae yellow (broken before midlength). Wings with a strong brownish yellow tinge, the prearcular and costal fields clearer yellow; stigma oval, pale brown; veins brown, clear yellow in the brightened fields. Abundant macrotrichia in apical cells of wing from Sc_2 to *1st A*, inclusive, especially numerous in radial and medial cells. Venation: Sc_1 ending about opposite fork of *Rs*, Sc_2 some distance from its tip, Sc_1 alone about three-fifths R_{2+3+4}; vein R_1 sinuous near the insertion of vein R_2; *Rs* long; R_{2+3+4} in longitudinal alignment with R_{2+3}; vein R_2 very faint to nearly obsolete, a little longer than R_{2+3}; cell M_1 small, about one-third its petiole; *m-cu* at from one-third to nearly opposite midlength of cell *1st* M_2.

Abdomen black, sparsely pruinose. Ovipositor with valves strong, yellowish horn colored.

Habitat: Northern Korea. *Holotype*, ♀, Kankyo Nando, Puksu Pyaksan, altitude 6000 feet, June 30, 1939 (Yankovsky).

The present fly is well-distinguished from all other regional members of the genus of major size. The most similar of such species is *Paradelphomyia* (*Oxyrhiza*) *majuscula* (Alexander), of western China. The present fly differs in the details of coloration and venation, as the short Sc_1, narrower cell R_3, and the unusually small cell M_1.

Genus **Archilimnophila** Alexander

Archilimnophila subunicoides n. sp.

Allied to *subunica;* antennae (male) relatively short, only about one-third as long as the wing; wings relatively narrow, restrictedly patterned with brown; male hypopygium generally as in *subunica*, the gonapophyses simple.

Male.—Length, about 9–10 mm.; wing, 9–10 mm.; antenna, about 3.2–3.3 mm.

Female.—Length, about 10–11 mm.; wing, 10.5–11 mm.

Rostrum gray; palpi brown. Antennae (male) relatively short for a member of the genus, dark brown; flagellar segments elongate, subequal to the verticils. Head gray; anterior vertex broad.

Pronotum gray, the sides of the scutellum obscure brownish yellow. Mesonotum gray, the praescutum with four more blackened stripes, the narrow intermediate pair only narrowly and incompletely separated by a gray vitta; scutal lobes weakly patterned with darker. Pleura gray, somewhat darker on the ventral sternopleurite; dorsopleural membrane buffy yellow to light brown. Halteres with stem obscure brownish yellow, knob more infuscated. Legs with the coxae yellow, more or less pruinose, the fore pair extensively more blackened, the middle pair similarly colored basally; trochanters yellow; fore femora extensively blackened, with about the proximal fourth or fifth yellow; remaining femora yellow, the tips more narrowly blackened; tibiae brownish yellow, the tips darkened; tarsi black. Wings relatively narrow, yellow, the base clearer yellow; a restricted brown pattern, including the stigma and clouds at origin of *Rs*, along cord, outer end of cell *1st* M_2 and fork of M_{1+2}; veins brown, darker in the patterned portions, clear yellow at wing base. Venation: R_{2+3+4} from about one-half to nearly two-thirds the strongly decurved R_4; cell M_1 usually subequal in length to its petiole, in cases a little longer or shorter; *m-cu* at from about one-fourth to beyond one-third the length of cell *1st* M_2.

Abdomen blackish, gray pruinose, including the hypopygium. Male hypopygium generally as in *subunica* but differing in important details. Outer dististyle a small strongly curved rod, the tip subacute. Inner dististyle with the yellow rostral portion long and slender, on outer margin close to base bearing a stout darkened lobe, its surface microscopically roughened. Each gonapophysis appearing as a slender sinuous blackened rod, very gradually narrowed to the acute tip, without a lateral branch, as in *subunica*.

Habitat: Northern Korea. *Holotype*, ♂, Puksu Pyaksan, altitude 4000 feet, June 5, 1939 (Yankovsky). *Allotopotype*, ♀, altitude 4200 feet, June 4, 1939. *Paratopotypes*, 7 ♂♀, altitude 3700–4500 feet, June 3–5, 1939 (Yankovsky).

Archilimnophila subunicoides is most closely related to the northern Nearctic *A. subunica* (Alexander), differing especially in the structure of the male hypopygium, as indicated above. The species is less similar to *A. harperi* (Alexander) and *A. unica* (Osten Sacken). I had formerly placed these flies in the genus *Austrolimnophila* Alexander but now believe that the genus *Archilimnophila* is separable though closely allied.

Genus **Limnophila** Macquart

Limnophila (**Prionolabis**) **clavaria** n. sp.

Size medium (wing, male, about 9 mm.); thorax brownish black, the surface opaque; legs black; wings broad, yellow, restrictedly patterned with darker; cell M_1 present but small; male hypopygium with the subtending lobe of the outer dististyle large, dark-colored, the style narrowed to an acute apical point, with a single obtuse lateral denticle; inner dististyle simple, with a blunt lobe at near three-fourths the length; gonapophysis appearing as a small straight darkened club, the tip blunt.

Male.—Length, about 8.5 mm.; wing, 8.8 mm.; antenna, about 1.6 mm.

Rostrum and palpi black. Antennae 16-segmented, black throughout; basal flagellar segments oval, the outer ones more elongate-oval; verticils of outer segments long and conspicuous. Head brown, opaque; anterior vertex broad.

Thoracic dorsum almost uniformly brownish black, the surface opaque by a sparse brownish yellow pollen. Pleura black, sparsely pruinose; dorsopleural membrane dark. Halteres yellow, the knobs a trifle more darkened. Legs with the coxae black, sparsely pruinose; remainder of legs black, the femoral bases very restrictedly brightened. Wings broad, ground color yellow, the prearcular and costal fields somewhat clearer yellow; stigma long-oval, brown; vague paler brown seams over anterior cord, vein R_5, the entire length of vein *Cu* and in the axillary portion of cell *2nd A;* veins dark brown, paler in the brighter yellow portions. Venation: Sc_1 ending just before the level of the fork of *Rs*, Sc_2 at its tip; R_{2+3+4} about three-fifths R_{2+3}; R_{1+2} a little longer than R_2; cell M_1 small, approximately one-half its petiole; *m-cu* at or just beyond midlength of cell *1st* M_2.

Abdomen, including hypopygium, black, the surface subnitidous, with black setae. Male hypopygium with the central region of the posterior border of the ninth tergite slightly produced and with a broad U-shaped notch, the lobes thus formed conspicuous. Outer dististyle with the subtending lobe large, dark-colored; style gradually narrowed to an acute curved apical point, with at most a single evident lateral denticle; outer margin with very long setae. Inner dististyle simple, the outer fourth slender, at point of narrowing with a blunt lobe, the apex of which is microscopically roughened or tuberculate. Aedeagus compressed-flattened, dark-colored. Gonapophysis appearing as a small straight darkened rod that widens very gradually outwardly, the apex obtusely rounded or weakly notched (in one apophysis of the unique type), the whole structure appearing as a small dusky club.

Habitat: Northern Korea. *Holotype*, ♂, Kankyo Nando, Puksu Pyaksan, Toorisani, altitude 6000 feet, June 22, 1939 (Yankovsky).

Limnophila (*Prionolabis*) *clavaria* is quite distinct from all other regional members of the subgenus, differing especially in the structure of the male hypopygium, as the dark-colored clavate gonapophyses. The fly is entirely different from the other species known from the Asiatic mainland.

Limnophila dis n. sp.

Allied to *yankovskiana;* general coloration black, including body and antennae; antennae of male elongate, exceeding one-half the length of wing; femora black, tibiae and tarsi dark brown; wings broad, with a strong blackish tinge; cell R_3 sessile or very short-petiolate; cell M_1 lacking; *m-cu* at or beyond midlength of cell *1st* M_2; male hypopygium with the outer dististyle a slender straight rod that is very shallowly and unequally bifid at tip, the longer spine being axial, the lateral branch much smaller.

Male.—Length, about 6–6.5 mm.; wing, 7–8 mm.; antenna, about 4.2–4.5 mm.

Rostrum and palpi black. Antennae of male elongate, as shown by the measurements; scape and pedicel black, flagellum dark brown;

flagellar segments apparently only 13 in number, elongate-cylindrical, with long coarse verticils and a shorter but conspicuous erect pubescence. Head dull black.

Thorax black, the surface subnitidous by a sparse pruinosity, pleura more heavily pruinose. Halteres infuscated, the extreme base of stem paler. Legs with the coxae black, very sparsely pruionse; trochanters dark; femora black, tibiae and tarsi dark brown or brownish black, the tips of the segments narrowly more darkened. Wings broad, with a strong blackish tinge; stigma oval, scarcely darker than the ground; scarcely indicated darker seams along the cord; veins brown. Venation: Sc_1 ending opposite fork of *Rs*, Sc_2 a short distance from its tip; *Rs* elongate, square and more or less spurred at origin; cell R_3 usually sessile, in cases barely so, with vein R_{4+5} punctiform or very short, in other cases, including the type, with R_{4+5} nearly as long as the basal section of R_5; in still other specimens, cell R_3 short-petiolate, with a short element R_{2+3+4} present; vein R_2 faintly indicated, about one-half R_{1+2}; cell M_1 lacking; *m-cu* opposite or shortly beyond midlength of cell *1st* M_2; vein *2nd A* moderately sinuous.

Abdomen, including hypopygium, black. Male hypopygium with the tergal region feebly emarginate. Outer dististyle a slender straight rod, very shallowly and unequally toothed at apex, the longer tooth axial, the shorter spine scarcely more than a tubercle, placed on mesal edge some distance from tip; inner dististyle unusually short and squat, the tip slightly decurved, obtuse.

Habitat: Northern Korea. *Holotype*, ♂, Puksu Pyaksan, altitude 5500 feet, June 13, 1939 (Yankovsky). *Paratopotypes*, 10 ♂♂, June 13–23, 1939 (Yankovsky).

Generally similar to *Limnophila yankovskiana* Alexander, likewise from the mountains of northern Korea, agreeing in the black coloration, elongate antennae of male, sessile or subsessile cell R_3, loss of cell M_1 and other characters, differing in the shorter antennae of male, more blackened wings, with the venational details, particularly of the medial field, distinct, and especially in the structure of the male hypopygium, notably the outer dististyle.

Limnophila pietatis n. sp.

General coloration of thorax reddish yellow, without pattern; antennae (male) elongate, the flagellar segments beyond the first uniformly black; femora yellow, the tips abruptly and very conspicuously blackened; wings with a weak brownish yellow tinge, restrictedly patterned with brown; *Sc* moderately long, Sc_1 ending before level of fork of *Rs*; R_{2+3+4} subequal to R_{2+3}; abdomen yellow, the anterior lateral angles of both tergites and sternites conspicuously blackened, in male, the outer three segments blackened; male hypopygium with the outer dististyle relatively short, glabrous, the apical outer angle produced into a slender fingerlike point; gonapophysis with seven or eight scattered spines on outer portion.

Male.—Length, about 7 mm.; wing, 6.8 mm.; antenna, about 3.2 mm.

Female.—Length, about 8.5 mm.; wing, 7.5 mm.

Rostrum orange yellow; palpi black. Antennae (male) elongate,

as shown by the measurements; basal three segments yellow, succeeding segments passing through dark brown to black; flagellar segments elongate-subcylindrical, the longest verticils a little shorter than the segments; in addition to the verticils, segments clothed with a dense erect pale pubescence. Head yellow, sparsely pruinose; anterior vertex broad, approximately four times the diameter of scape. In the female referred to this species, the antennae are shorter, the scape blackened; head above much clearer gray.

Entire thorax uniformly reddish yellow, subnitidous, without pattern. Halteres relatively long, stem yellow, knob very slightly darker. Legs with the coxae and trochanters yellow; femora yellow, the tips rather broadly and abruptly black, occupying about the outer seventh or eighth of segment; tibiae paler yellow, the base diffusely infuscated, the tip more narrowly and abruptly blackened; basitarsi and second tarsal segment almost white, the tips darkened; outer tarsal segments brownish black. Wings with a weak brownish yellow tinge, the prearcular and costal fields clear light yellow; a restricted brown pattern, including stigma, a large cloud at origin of *Rs*, cord, outer end of cell *1st* M_2, and less evidently elsewhere; outer radial cells even less distinctly infuscated; veins brown, yellow in the brightened areas. Venation: *Sc* moderately long, Sc_1 ending before fork of *Rs*, Sc_2 even longer, opposite this fork; *Rs* elongate, square and in cases, including the type, long-spurred at origin; R_{2+3+4} relatively long, about equal to R_{2+3}, in direct longitudinal alignment with *Rs;* R_{1+2} and R_2 subequal; inner ends of cells R_4, R_5 and *1st* M_2 in transverse alignment; cell M_1 from one-third to one-half longer than its petiole; cell *1st* M_2 rectangular, with *m-cu* at from one-third to two-fifths its length; vein *2nd* *A* long, generally paralleling the axillary margin of wing.

Abdomen yellow, the anterior lateral angles of both tergites and sternites two to four, with a conspicuous black area that narrows behind, extending to beyond midlength of segment, on the succeeding segments becoming smaller and less evident, more distinct in female; male with terminal three segments black, only the hypopygium partly brightened; in female, the pale color continued virtually to the end, including the genital shield. Male hypopygium with the outer dististyle relatively short, appearing as a gently curved flattened blade, at end with apical outer angle produced into a slender fingerlike point; surface glabrous except for a single strong seta at basal third. Inner dististyle a little shorter, the basal half or more oval, the outer portion more narrowed; surface of style with conspicuous erect setae. Gonapophysis provided with seven or eight conspicuous acute points distributed over the entire head or apical portion.

Habitat: Northern Korea. *Holotype*, ♂, Puksu Pyaksan, altitude 5000 feet, August 21, 1939 (Yankovsky). *Allotopotype*, ♀, pinned with type. *Paratopotypes*, 3 ♂♂, August 9–21, 1939 (Yankovsky).

Very similar in its general appearance to the western Palaearctic *Limnophila glabricula* (Meigen), of which it may prove to represent a race. The dististyles of the male hypopygium are conspicuously shorter than in *glabricula*. Edwards referred *glabricula* to the subgenus *Phylidorea* Bigot but its position here must still be held as questionable.

Genus **Lipsothrix** Loew

Lipsothrix yamamotoana n. sp.

General coloration dull black; antennae black, relatively long, if bent backwards extending approximately to the wing-root, the flagellar verticils shorter than the segments; femora yellow, the tips brownish black, slightly more extensive on the fore legs where about the outer fifth is included; wings yellowed, the prearcular and costal fields clearer yellow; *Sc* short, Sc_1 ending about opposite the fork of *Rs; Rs* relatively long, exceeding four times R_{2+3+4}, the latter shorter than R_{2+3}; inner end of cell R_4 lying only a little proximad of the other elements of the anterior cord; *m-cu* close to the fork of *M*.

Male.—Length, about 7–7.5 mm.; wing, 8–8.5 mm.; antenna, about 2.1–2.3 mm.

Rostrum and palpi black. Antennae black, relatively long, if bent backward extending approximately to the wing-root; flagellar segments oval, the constrictions between the segments well indicated; verticils shorter than the segments. Head dull black.

Thorax uniformly dull black. Halteres pale yellow. Legs with the coxae and trochanters yellow; femora yellow, the tips brownish black, slightly more extensively so on the fore legs where about the outer fourth or fifth is included; tibiae obscure brownish yellow, the tips narrowly darkened; tarsi brown; claws (male) toothed. Wings with the ground color yellow, the prearcular and costal fields clear light yellow; stigma oval, brown, relatively inconspicuous; veins brown, yellow in the brightened areas, especially at wing base. Venation: *Sc* short, Sc_1 ending about opposite fork of *Rs*, Sc_2 at its tip; *Rs* relatively long, exceeding four times R_{2+3+4}, the latter shorter than R_{2+3}; R_{1+2} very short; inner end of cell R_4 lying only a short distance proximad of the other elements of the anterior cord; cell *1st* M_2 rather short-rectangular; *m-cu* close to or just beyond the fork of *M*.

Abdomen, including hypopygium, black, sparsely pruinose.

Habitat: Japan (Honshu). *Holotype*, ♂, Funakosi, Iwateken, altitude 200 meters, May 23, 1947 (H. Yamamoto). *Paratopotype*, ♂, pinned with the type.

I am very pleased to name this distinct fly for the collector, Mr. Hiromu Yamamoto, to whom I am greatly indebted for many interesting Tipulidae from northern Honshu. From other regional members of the genus, including *Lipsothrix tokunagai* Alexander and *L. yakushimae* Alexander, it is readily told by the coloration and by the structure of the antennae and the venation.

Genus **Gnophomyia** Osten Sacken

Gnophomyia (**Gnophomyia**) **acheron** n. sp.

General coloration black; antennae black, the flagellar segments in male relatively long, subcylindrical; wings with a weak blackish tinge, the prearcular field light yellow, stigma not indicated; Sc_1 unusually long; ovipositor with cerci unusually long and slender, gently upcurved; basistyle of male hypopygium with a group of six or seven stout black

setae on mesal face; outer dististyle a long, nearly straight blackened rod, the tip obtuse.

Male.—Length, about 7.5–8 mm.; wing, 7.5–8 mm.; antenna, about 2–2.1 mm.

Female.—Length, about 8 mm.; wing, 8 mm.

Rostrum and palpi black. Antennae black, the scape a little more pruinose; flagellar segments in male relatively long, subcylindrical, longer than the verticils. Head dull black, the anterior vertex broad, heavily dusted with gray.

Thorax black, subopaque, the ventral pleurites more distinctly pruinose. Halteres pale yellow. Legs with the coxae and trochanters black; remainder of legs black, the femoral bases very narrowly yellowed, most evident on the fore legs. Wings with a weak blackish tinge, the prearcular field light yellow; stigma not indicated; veins dark brown. Venation: Sc_1 ending approximately opposite R_2, Sc_2 nearly opposite the fork of Rs, so Sc_1 is unusually long, subequal to or even exceeding the combined veins R_{2+3+4} and R_{2+3}; veins R_3 and R_4 extending generally parallel to one another, slightly divergent; cell *1st* M_2 shorter than vein M_4, with *m-cu* at near one-third its length.

Abdomen, including hypopygium, black. Ovipositor with the cerci unusually long and slender, gently upcurved. Male hypopygium with the region of the ninth tergite with a shallow emargination, the lobes very broad, at base of notch with a few short strong spines. Mesal face of basistyle with a group of six or seven stout black setae on mesal face. Outer dististyle a long, nearly straight blackened rod, its tip obtuse. Inner dististyle much smaller, broad at base, the outer half strongly narrowed and upturned, on dorsal face of the enlarged part with a compact group of about eight long black spinous setae. Phallosome massive, subquadrate in outline, the caudal margin very gently emarginate medially.

Habitat: Japan (Honshu). *Holotype*, ♂, Funakosi, Iwateken, altitude 200 meters, May 23, 1947 (H. Yamamoto). *Allotopotype*, ♀. *Paratopotypes*, 6 ♂ ♀.

The only approximately similar regional species is *Gnophomyia* (*Gnophomyia*) *nycteris* Alexander, still known to me only from the female. This differs in the details of coloration and venation and especially in the structure of the ovipositor.

Genus **Ormosia** Rondani

Ormosia (Ormosia) weymarni n. sp.

Mesonotum brownish gray, the postnotum and pleura yellow; antennae short; halteres with knobs dusky; legs yellowish brown, the tips of the femora and tibiae narrowly darkened; wings grayish yellow, the prearcular and costal fields clearer yellow; macrotrichia of cells long and conspicuous but relatively sparse, more or less restricted to the central portions of the cells; phallosome unblackened.

Male.—Length, about 4–4.2 mm.; wing, 4.4–4.8 mm.

Rostrum and palpi pale. Antennae short, if bent backward extending about to mid-distance to wing root or slightly beyond; scape and

pedicel light brown, flagellum black; flagellar segments oval, the verticils a little exceeding the segments. Head dark gray.

Pronotum obscure yellow. Mesonotal praescutum brownish gray, without clearly defined stripes; scutum and scutellum brownish gray, the postnotum yellow. Pleura obscure yellow. Halteres dusky, the basal half of stem yellow. Legs with the coxae and trochanters yellow; femora yellowish brown, the bases clearer yellow, the apices passing into brown; tibiae obscure yellow, the tips narrowly infuscated; tarsi brownish black. Wings grayish yellow, the prearcular and costal fields clearer yellow; veins pale brown, more yellowed in the brightened costal portions. Macrotrichia of cells relatively long but sparse, lacking in the basal portions of cells *R*, *M* and *Cu;* in the other cells more or less restricted to the central parts of the cells remote from the veins. Venation: Sc_1 ending about opposite fork of *Rs*, Sc_2 about opposite one-fifth to one-sixth the length of *Rs;* cell M_2 open by the atrophy of the basal section of M_3; *m-cu* just before the fork of *M;* Anal veins divergent.

Abdominal tergites darkened medially, paler on sides; sternites and hypopygium yellow. Male hypopygium with the outer dististyle relatively small, provided with rows of scabrous blackened points; inner dististyle subequal in length, pale. Phallosome appearing as tumid, entirely unblackened plates that are contiguous on the midline.

Habitat: Manchuria, northern Korea. *Holotype*, ♂, Kaolingtze, Manchuria, May 30, 1941 (received from Michael Weymarn). *Paratype*, ♂, Ompo, northern Korea, altitude 600 feet, May 2, 1938 (Yankovsky).

This interesting fly is named for Mr. Michael Weymarn, to whom I am indebted for many Tipulidae from Manchuria. Despite the different venation, the fly seems closest to *Ormosia* (*Ormosia*) *confluenta* Alexander, of Japan, and *O.* (*O.*) *yankovskyi* Alexander, of northern Korea, both of which have cell M_2 of the wings open by the atrophy of *m* and differ further in the color of the body and legs and in the details of structure of the male hypopygium. Other allied species in the northwestern Nearctic fauna include *O.* (*O.*) *absaroka* Alexander, *O.* (*O.*) *flaveola* (Coquillett), and others.

Genus **Molophilus** Curtis

Molophilus (**Molophilus**) **hoplostylus** n. sp.

Belongs to the *gracilis* group; general coloration blackened; antennae of male elongate, approximately two-thirds the body, black throughout; flagellar segments fusiform, with whorls of long erect setae at midlength; wings broad, suffused with blackish; costal fringe long and dense; vein *2nd A* short; male hypopygium with three dististyles, the longest a gently curved black rod with numerous denticles along the face; third dististyle a small fingerlike fleshy lobe that bears numerous very long setae.

Male.—Length, about 5 mm.; wing, 4.8 mm.; antenna, about 3.5 mm.

Rostrum and palpi black. Antennae (male) elongate, as shown by

the measurements, black throughout; flagellar segments fusiform, with whorls of very long erect setae at midlength of the individual segments. Head black.

General coloration of thorax black, probably pruinose in fresh specimens, the unique type discolored; pretergites restrictedly yellow. Halteres black. Legs with the coxae and trochanters brown; remainder of legs dark brown. Wings broad, with a weak blackish tinge; veins and macrotrichia brownish black; costal fringe (male) long and dense. Venation: R_2 in approximate transverse alignment with ***r-m; m-cu*** less than one-third the length of the petiole of cell M_3; vein ***2nd A*** unusually short, ending some distance before the level of ***m-cu***.

Abdomen, including hypopygium, black. Male hypopygium complex; apex of basistyle with a cylindrical dorsal lobe that is provided with long retrorse setae, and a longer ventral lobe that is dilated on the basal two-thirds or more, provided with long erect setae, the apical part slender and glabrous, the tip obtuse. Three dististyles, the longest a gently curved black rod that narrows very gradually into a long straight spine, the margin, especially the lower or concave one, with microscopic denticles, one at near midlength longer; second dististyle nearly as long, straight, the outer third a more slender blackened spine; third dististyle a small fingerlike fleshy lobe that bears numerous very long setae, these longer than the style itself. Phallosomic plate obtuse at tip, the surface with exceedingly microscopic setulae. Aedeagus elongate, subtended by a flange, the apex more or less bilobed.

Habitat: Formosa. *Holotype*, ♂, Musha (Wuse), Telchung District, altitude 1000 meters, August 23, 1947 (J. L. Gressitt); type in the Lingnan University Collection.

The present fly belongs to a subgroup having rather numerous species in the Philippines and elsewhere in southeastern Asia, but to this date with no known representatives in Formosa or northward. The most similar Philippine species include ***Molophilus*** (***Molophilus***) ***banahaoensis*** Alexander, ***M.*** (***M.***) ***hispidulus*** Alexander, and ***M.*** (***M.***) ***injustus*** Alexander, all differing from the present fly, and among themselves, in the structure of the male hypopygia.

FORMIC ACID PRODUCTION AMONG THE FORMICIDAE

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INTRODUCTION

Although it has been known for a long time that ants secrete formic acid there are very few papers on the subject which are accompanied by analytical data. Poulton (1901), in a brief note, described an apparatus which could be used to collect the venom of ants. He stated that the percentage of anhydrous acid in the venom of *Formica rufa* fluctuated widely and that strong samples contained 60% to 70% of acid. Melander and Brues (1906) estimated formic acid by the reduction of mercuric chloride, but they found that simple titration with N/2 potassium hydroxide after steam distillation from weak ethyl alcohol gave more consistent results. Sexual forms never gave an acid reaction with the exception of *Formica* females. These authors made the extremely important observation that only the genera not having stings secrete acid. *Crematogaster* (Myrmicinae) they say "may prove to be an exception, but in this case most likely some other acid is present." *Forelius* (Dolichoderinae) contained no acid but *Pachycondyla* (Pomerinae), although provided with a powerful sting, contained acid up to 1.5% of its body weight. *Formica fusca* var. *gnava* workers contained acid to the extent of from 0.5% to 12.68% of their body weight. Stumper (1922a), using Duclaux's method for estimating formic acid, found that it was the only free volatile acid present in the venom of *Formica rufa* and *Cataglyphis bicolor*. Later (1922b) the same author showed that the venom of *Formica rufa* contained from 21% to 72% (*i.e.*, 5–17M) of formic acid. He also showed that temperature affected the rate of secretion and his results gave a Q^{10} of 2.16. He pointed out that the tissues of the poison sac and the ejaculatory duct resist the action of such a strong acid and that the mechanism of that resistance is quite unknown. Donisthorpe (1927) gave results obtained by Briscoe using *Formica rufa* workers which produced from 1.8 to 2.1 mg. of acid per ant. It will be seen, therefore, that the production of formic acid is limited to one subfamily of the Formicidae, with only two exceptions—*Pachycondyla* and *Crematogaster*. Both these are inconclusive since it was only shown that an acid was present and there is no evidence as to it being formic acid. Thus, so far as is known, the Formicidae alone among the ants secrete formic acid. This sub-

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[2]I am grateful to Dr. B. P. Beirne, of Trinity College, Dublin, and Mr. W Crewe, of the Department of Entomology and Parasitology, Liverpool School of Tropical Medicine, who criticised the manuscript; and to Dr. T. G. Brady, of the Department of Biochemistry, University College, Dublin, who made a number of helpful suggestions.

family is characterized by the rudimentary nature of the sting and the marked development of a venom sac in which the formic acid is stored.

Stumper (1923) was the first to point out the association between the presence of the poison apparatus and well-developed glands with the rudimentary nature of the sting and the secretion of formic acid. He showed that while the Formicinae always secrete formic acid, the Dolichoderinae and the Myrmicinae never do so.

Many authors quote figures unsubstantiated by either references or experiments; for example, Ceballos (1943, p. 331) states "The poison of these ants (Formicinae) show concentrations of formic acid as high as 72% although that of the species of other subfamilies contain no formic acid." (Translation). I have been unable to find any other papers based on analytical work. It will therefore be seen that, as yet, there is little known about formic acid secretion and that there is a wide field for research.

PRESENT WORK

(a) *Titration method*

In the experiments described here an attempt was made to estimate the amount of acid present in each species available. The procedure was as follows:

Single ants were weighed on a micro balance and used for each estimation which was done on a micro-chemical scale. The acid was estimated by titration with $\frac{N}{200}$ NaOH with phenolphthalein (one drop) as indicator. $\frac{N}{800}$ NaOH was used in early experiments but it was found that this solution lost its strength rather rapidly because of absorption of CO_2 from the atmosphere. A single ant was taken alive, weighed and dropped into one ml. of distilled water which was then brought to the boil. A further ml. of water was then added to wash the sides of the test-tube and wash back the slight condensation vapour. One drop of phenophthalein was then added and the solution titrated from a Bang burette with the alkali until it became colourless. It was found that a blank titration was necessary and subtraction of the blank made a considerable difference to the result. In the tables which follow, I give in brackets after the percentage of body weight these uncorrected figures. By chance a simple method for detecting minute amounts of acid was discovered when an ant fell into a drop of B.D.H. soil indicator and turned the indicator from green to bright red. It was found by experiment that using one drop of indicator as little as 1.1γ of HCOOH (1γ being one millionth of a gram) could be detected. This method was used to test all available species. Tables I, II, and III, show the results obtained.

These are the only acid-containing species found in this country and which, as a result, I have been able to use for estimation.

Leptothorax acervorum, and all the Irish species of *Myrmica* except *schenki* have been tested with negative results. These figures are interesting in that they are the first set giving complete data and using

TABLE I

FORMIC ACID CONTENT OF FORMICA FUSCA WORKERS

Specimen No.		Weight of Specimen in Micrograms	Weight of Acid in Micrograms	Percentage of Total Body Weight
Workers tested within 24 hours of collection	1	2,900	106	3.96 (4.96)
	2	4,200	0	0 (0.64)
	3	3,500	0	0 (0.80)
	4	4,100	76	1.85 (2.75)
	5	3,100	0	0 (0.68)
	6	10,000	57	0.57 (0.94)
	7	12,100	27	0.22 (0.53)
	8	9,200	30	0.32 (0.73)
	9	10,000	57	0.57 (0.94)
	10	12,200	71	0.58 (0.89)
Eight workers collected 3 days previously		40,200 (total)	5,219	1.29
Ten workers dead one month		35,000 (total)	2,182	6.31
Average				0.81

TABLE II

FORMIC ACID CONTENT OF LASIUS FLAVUS WORKERS

Specimen No.		Weight of Specimen in Micrograms	Weight of Acid in Micrograms	Percentage of Body Weight
Alive	1	900	60	6.66 (10.8)
	2	800	0	0 (4.7)
	3	2,200	4.1	1.86 (3.5)
Dead 24 hours	4	1,100	69	6.27 (9.2)
	5	1,700	16	0.94 (3.1)
	6	1,100	2	0.18 (3.5)
Average				2.65

TABLE III

FORMIC ACID CONTENT OF LASIUS NIGER WORKERS

Specimen No.		Weight of Specimen in Micrograms	Weight of Acid in Micrograms	Percentage of Body Weight
Dead less than 12 hours	1	2,100	88	4.19 (5.9)
	2	2,100	3	0.14 (2.8)
	3	2,600	389	14.96 (16.4)
	4	2,000	239	11.95 (13.8)
	5	1,500	106	7.07 (9.5)
	6	2,400	191	7.96 (9.5)
Average				7.71

single specimens. The following observations may be made on these figures:

(1) While the figures compare very favourably with other available figures, it will be noticed that they tend to the lower than those hitherto published. It is possible that this is due to the fact that in the present experiments care was taken to do control experiments. In all cases the results have been used in the subsequent calculations of the percentage of formic acid in the body.

(2) It will be noted that there is a wide scatter in the results. This might be due to the fact that one might be dealing in one case with a specimen whose poison gland was full, and in another with one whose gland has just been emptied.

(3) One may note the high values obtained with specimens of *L. niger*, which were dead. In general dead specimens gave higher values than did living ones; a fact which may account for the higher percentages obtained by previous experimenters, who do not state whether the ants were alive or not when they began the determinations.

It will be seen that the method used depended merely on the acidic properties of formic acid. It is possible that there are other titratable acids in ants as well as formic acid. It was, therefore, decided to try to devise a quantitative method that would be specific for formic acid. The following methods were tried.

(b) *Microdiffusion Method*

This method, based on the liberation of bromine, made use of the Conway Unit in the outer chamber of which 1 ml. of the formic acid was placed. The central chamber contained 1 ml. of a standard sodium hypobromite solution.[3] One ml. of normal sulphuric acid was added to the outer chamber and the units sealed with a beeswax-paraffin fixative and incubated at 50° C. for 24 hours. The units were then allowed to cool and the sodium hypobromite sucked out into a tube and diluted with 1 ml. distilled water and acidified with 10% hydrochloric acid until the bromine just appeared. The tube was then stoppered and left in the dark for 30 minutes when it was saturated with potassium iodide. It was then titrated with $\frac{N}{20}$ thiosulphate from a Conway burette. The difference between this titration and that for the blank unit gave the amount of formic acid. 1 ml. $\frac{N}{20}$ thiosulphate being equivalent to 1.15 mg. formic acid.[4]

[3]This was made up as follows: 15 grams of pure sodium hydroxide was dissolved in 450 ml. of distilled water in a 500 ml. volumetric flask. When cold 5 ml. of bromine was added and dissolved by shaking. The whole was then made up to 500 ml. and kept in a dark stoppered bottle in the dark. The solution was standardized by diluting 5 ml. of it with 50–70 ml. of water and adding 20 ml. of 10% hydrochloric acid and 1 gram of potassium iodide. After two minutes the iodine liberated was titrated with $\frac{N}{20}$ thiosulphate.

[4]Reference to (1) microdiffusion method in Conway 1947, and (2) to the chemical basis of the method in Rupp 1905, and Thorpe and Whiteley 1925.

It was not expected that all the formic acid would diffuse across into the central chamber but (in a series of experiments) the yield proved to be very poor, only 29% of the formic acid coming across. The sodium hypobromite in the central chamber was replaced by potassium hydroxide, the former then being added after cooling. The results, however, continued to be both erratic and low although the blanks became fairly constant indicating that the method rather than the technique was at fault. It is probable that higher incubation temperatures (about 80° C.) should have been used, but at these temperatures the fixative is difficult to control.

(c) *Colorimetric Method*

The principle was to reduce the formic acid to formaldehyde with magnesium in the acid medium. The formaldehyde was then allowed to react with 0.5 ml. of 1% phenylhydrazine in 2% hydrochloric acid and 0.05 ml. of 5% potassium ferricyanide until the colour was fully developed when it was estimated with Pulfrich photometer using filter 9 and 20 mm. stratum length. This reaction gave a good colour which however showed marked fading. An endeavour to overcome this using different reaction times eventually gave a good extinction curve, using standard formic acid solutions. When, however, ant extracts were used the fading was uncontrollable and after many attempts the method had to be abandoned. (The method will detect as little as 50γ of formic acid and is useful qualitatively.)

The present method gave a colour reaction with *Formica fusca*, *Lasius niger* and *L. flavus* but none with any species of *Myrmica* thus confirming the presence of formic acid in formicine species, and its absence from myrmicine ones.

SYNTHESES OF FORMIC ACID IN ANTS

Nothing is known of the method of synthesis of the acid in the ants. The best that can be done is to suggest the possible modes which might be used by the organism for this synthesis.

I. $CO_2 + H_2O \rightleftharpoons HCHO + O_2$. This is the path suggested for the carbohydrate synthesis in plants, the formaldehyde then being polymerized to monosaccharides by aldol condensations. If we then assume oxidation of the formaldehyde formic acid would result:

$$2\,HCHO + O_2 \rightleftharpoons 2HCOOH.$$

It has recently been shown (cf. Rabinowitch (1945), chapter 4) that the formation of formaldehyde does not account for carbohydrate synthesis in plants so that the probability of this mode of formation is reduced. A point in its favour is that the increased CO_2 production during activity, which is known to occur in insects (Wigglesworth, 1942: 341), would find an outlet in acid production since the greatest amount would be required under such circumstances. The possibility of this mode of synthesis could be tested by bubbling CO_2 and O_2 through an extract of ants and testing for formaldehyde with dimedone. This could not be carried out as no supplies of dimedone were available.

II. Bersin (1938) quotes the results obtained by Stephenson and Stickland with *Bacillus lactis aerogenes* and *B. coli*, in which formic

acid is built up from hydrogen and carbon dioxide by means of the enzyme formic acid dehydrase

$$CO_2 + H_2 \rightleftarrows HCOOH$$

the H_2 coming from a hydrogen donator. This is certainly a possible line of synthesis although the enzyme hydrogen lyase has not been found in any group except bacteria. Now that it is known that symbiotic bacteria produce the vitamins required by insects, and that, furthermore, such symbionts are known in ants of the genera *Formica* and *Camponotus* (cf. Wigglesworth, 1942: 287 ff.), it is by no means impossible that such organisms could form the acid. It is difficult to imagine that the bacteria could produce enough acid at the time required by the insect. Thus a metabolic origin is *a priori* the most likely since acid production would be correlated with the needs of the insect.

III. Formic acid might result as a byproduct of carbohydrate metabolism. Although there are many points at which formic acid might be split off there is nothing to support any of these possibilities. A possible point in the cycle at which the formic acid could come off is at the oxaloacetic acid stage. Instead of it being converted into the enol form and combined with the keto form of pyruvic acid to form oxalocitraconic acid in the oxidative breakdown of glycogen known to occur in insects (cf. Wigglesworth, 1942: 329) the oxaloacetic acid might split off formic acid on hydrolysis, thus:

$$\begin{array}{l} \text{COOH} \\ | \\ \text{CO} \\ | \\ \text{CH}_2 \\ | \\ \text{COOH} \end{array} + \text{HOH} \rightleftarrows \begin{array}{l} \text{COOH} \\ | \\ \text{CO} \\ | \\ \text{CH}_2\text{OH} \end{array} + \text{HCOOH}$$

The former compound by picking up a hydrogen molecule would give rise to the familiar pyruvic acid in the keto form; as follows:

$$\begin{array}{l} \text{COOH} \\ | \\ \text{CO} \\ | \\ \text{CH}_2\text{OH} \end{array} + H_2 \rightleftarrows \begin{array}{l} \text{COOH} \\ | \\ \text{CO}\ H_2O \\ | \\ \text{CH}_3 \end{array}$$

Another possible method is by the hydrolysis of ketoglutaric acid instead of the usual simple oxidation, thus:

$$\begin{array}{l} \text{COOH} \\ | \\ \text{CH}_2 \\ | \\ \text{CH}_2 \\ | \\ \text{CO} \\ | \\ \text{COOH} \end{array} + H_2O \rightleftarrows \begin{array}{l} \text{COOH} \\ | \\ \text{CH}_2 \\ | \\ \text{CH}_2 \\ | \\ \text{COOH} \end{array} + \text{HCOOH}$$

succinic acid

This is quite a likely method.

IV. Finally, the possibility of a derivation from amino acids cannot be ignored. Glycine $CH_2.NH_2COOH$, which occurs to the extent of 3.5% in the silkworm moth (Wigglesworth, 1942: 327) could form formic acid by deamination with the consequent oxidation of formaldehyde thus:

$$\begin{array}{lcl} H_2-C.COOH & & H_2-C.COOH \\ | & & || \\ NH_2 & \xrightleftharpoons{\text{glycine deaminase}} & O \end{array} \quad +H_2O \rightleftharpoons HCOOH+H_3CO_2$$

If glycine deaminase is found in ants it would furnish strong support for this possibility.

It was considered worth while outlining these possibilities, although at present I have no evidence to support any of these hypotheses which, nevertheless, have been stated. It is hoped that in the future I may have the time and facilities, not now at my disposal, to test out the theories which are worthy of further investigation.

It will be seen that there is still a great deal to be done in the present field. The main difficulty is to find a reliable method of estimating micro-quantities of formic acid in ants.

SUMMARY

1. Previous work is reviewed and the problems yet to be worked out are indicated.

2. The results of formic acid estimations using a titration method are given, and they are shown to be lower than those previously recorded. It is suggested that this may be because no blank estimations were made by previous workers, and also that they used dead specimens.

3. An unsuccessful attempt to produce a reliable method of formic acid estimation on a micro-diffusion basis is described. A qualitative colorimetric method is described which will detect as little as 50γ of formic acid. It did not prove to be useful as a quantitative method.

4. Four possible modes of formic acid synthesis in the ant are suggested and discussed.

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DEGRADATION OF DDT BY RESISTANT AND SUSCEPTIBLE STRAINS OF HOUSE FLIES[1]

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It has been demonstrated by Sternburg *et al*, (1950) and Perry and Hoskins (1950) that certain strains of DDT-resistant house flies have an abnormal facility to degrade DDT to its non-toxic metabolite DDE (1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethylene). The difference between susceptible and resistant strains is so great in this respect that it would seem to be a major factor in the explanation of the nature of DDT-resistance. It seemed that further studies on the metabolism of DDT by the two strains of flies might lead to a clarification of the significance of DDT-degradation in relation to the acquisition of DDT-resistance and at the same time contribute much needed information on the mode of action of DDT. The previous work indicated the need for information concerning the site or sites at which DDT is degraded in resistant flies. It also seemed desirable to extend the studies on DDT metabolism to stages other than the adult.

The strains of flies, methods of rearing, extraction and analysis of DDT and metabolites, and application of chemicals to insects were for the most part the same for the present report as those described by Sternburg *et al*, (1950). In any instance where changes have been made they are fully described.

SITE OF DDT DEGRADATION

Preliminary attempts to locate the sites of DDT degradation in resistant flies consisted in the analysis of ether extracts of gross tissue dissections from adult flies which had been previously treated with DDT. The object was to locate areas of the body where the metabolite DDE accumulated in greatest quantity and then to explore those particular areas in greater detail with respect to the nature of the tissues associated with its accumulation and degradation. Twenty DDT-resistant flies were each treated with 10 μg. of DDT applied to the mesonotum in 1 $mm.^3$ of ethanol. They were then held for 24 hours in a cheesecloth-covered erlenmeyer flask, after which time they were rinsed with ether to remove DDT from the body surface. The flies were then frozen until they could be dissected. The tissues dissected from the flies were not free from contamination of other materials such as blood and fat bodies. No attempt was made to dissect tissues from the appendages, and the tissues designated as cuticle actually consisted of cuticle, hypoderm, and stubs of muscle at their points of attachment to the cuticle. The designations given in Table I, therefore, indicate the greater proportion of the particular tissue which was assayed for DDT and its metabolites. The total of all tissues analyzed represents the analysis of a whole fly.

[1]Cost of publication paid by the University of Illinois.

The data recorded in Table I show that approximately three-fourths of the total dosage of 200 μg. of DDT applied to the 20 flies remained on the outside of the flies and was readily recovered in the ether rinse as unmetabolized DDT. The remainder of the total dose, approximately 50 μg., could be partially accounted for by the total of 34.5 μg. of DDE recovered from various tissue extracts. The recovery of DDT and its metabolite DDE is unexpectedly good in view of all the manipulations involved, and the fact that the pooled fly tissues in some cases represented such a small amount that they could have contained DDT and metabolites in quantities too small to assay.

TABLE I

RECOVERY OF DDT AND DDE FROM POOLED TISSUES FROM RESISTANT FLIES 24 HOURS AFTER TOPICAL TREATMENT WITH 10 μg. DDT TO THE MESONOTUM OF EACH FLY

TISSUE ANALYZED*	RECOVERY PER FLY	
	μg. DDE	μg. DDT
Cuticle of head	0.10	0.00
Cuticle of thorax	0.32	0.00
Cuticle of abdomen	0.58	0.00
Legs and wings	0.20	0.00
Brain and thoracic ganglion	0.00	0.00
Thoracic muscles	0.28	0.00
Fore gut	0.00	0.00
Mid gut	0.15	0.00
Hind gut	0.10	0.00
Mal. tubules	0.00	0.00
Sex organs	0.00	0.00
Total amount, internal	1.73	0.00
External body surface	0.0	7.53
Total recovered based on molecular wt. of DDT		9.45

*Total of all tissue assayed equals analysis of whole insect.

The significant features of the data in Table I are that DDE may be found in most of the areas of the body but is most abundant in cuticle of the flies in the regions of the body where the dosage was applied. This suggests the possibility that DDT may be degraded to DDE by resistant flies during the time it is being absorbed through the cuticle-hypoderm. The fact that some DDE was found in or on the intestinal tract suggested the possibility that the flies might be getting small oral doses of DDT by sponging the contaminated surfaces of other flies or the flask in which they were held.

These observations led to a second experiment in which the mouth parts, legs, and wings of 20 resistant flies were removed before treatment with DDT at a dosage of 10 μg. per fly. The flies were then dissected into the various fractions indicated in Table II and analyzed

for DDT and metabolites at various intervals after application of the compound. The flies treated in this manner remained alive for as long as 24 hours after treatment.

The recovery of DDT and metabolites from the treated flies ranged from 76 to 90 per cent of the theoretical quantity. The loss is probably accountable for largely by mechanical means such as contamination of equipment used in handling and dissecting the flies. Two additional causes for loss may be from the metabolism of DDT to form products which do not respond to the analytical method of Schecter *et al*, (1945), and the probability that some of the tissues contained DDT or

TABLE II

RECOVERY OF DDT AND DDE FROM POOLED TISSUES DISSECTED FROM GROUPS OF 10 RESISTANT FLIES AT VARIOUS TIMES AFTER THEY WERE TREATED TOPICALLY WITH 10 μG. DDT PER FLY AFTER REMOVAL OF MOUTHPARTS, LEGS, AND WINGS

MATERIAL ASSAYED*	μG. OF DDT AND DDE RECOVERED PER FLY					
	4 Hours		8 Hours		24 Hours	
	DDT	DDE	DDT	DDE	DDT	DDE
Internal:						
Entire head	0.00	0.00	0.00	0.10	0.00	0.33
Thoracic cuticle	0.00	0.35	0.14	0.22	0.16	0.66
Thoracic muscle	0.00	0.00	0.00	0.00	0.00	0.00
Abdominal cuticle	0.00	0.10	0.00	0.20	0.00	0.41
Abdominal gut	0.00	0.00	0.00	0.00	0.00	0.00
Total internal	0.00	0.45	0.14	0.52	0.16	1.40
External body surface	8.30	0.00	6.90	0.00	7.25	0.00
Total recovered based on molecular wt. of DDT	8.80		7.62		8.96	

*Total of all tissue assayed equals analysis of whole fly.

metabolites in amounts too small to determine. It is significant, however, that detoxification of DDT applied topically to resistant flies may occur in the cuticle-hypoderm of the body area covered by the treatment. The fact that neither DDT nor DDE was found in any of the internal tissues of the flies in measurable quantities suggests that either DDT produces its affects by interfering with the physiology of the peripheral body areas or only a minute fraction of the topical lethal dose is required to be centrally distributed in the insect. It is of interest to note that where the mouth-parts are removed before treatment that DDE does not appear in the intestinal tract and other tissues of the body. It is assumed, therefore, that the presence of DDE found in these areas of the body (Table I) was due to the flies taking up DDT orally from contaminated surfaces.

The possibility that resistant flies are able to degrade DDT by means of the digestive tract led to the next experiment in which 20 resistant

flies were allowed to feed on milk containing 2 mg. of DDT per ml. of milk. The flies were permitted to feed until satisfied and then held for 15 hours without further feeding. They were then dissected and analyzed for the presence of DDT and metabolite in various tissues. The results, shown in Table III, indicate that degradation of DDT can occur in the digestive tract, as well as in the cuticle-hypoderm. DDE formed in the digestive tract apparently is transported to all parts of the body, whereas non-metabolized DDT is retained in the digestive tract and does not accumulate in other parts of the body. The ability of resistant flies to degrade oral dosages of DDT before any has reached a vital site thus appears to be a further factor in resistance to DDT.

TABLE III

RECOVERY OF DDT AND DDE FROM POOLED TISSUES DISSECTED FROM 20 RESISTANT FLIES 15 HOURS AFTER FEEDING ON MILK CONTAINING 2 MG. DDT PER ML.

TISSUE ANALYZED*	RECOVERED PER FLY	
	μg. DDT	μg. DDE
Cuticle, head	0.00	0.35
Cuticle, thorax	0.00	0.85
Cuticle, abdomen	0.00	1.09
Brain and thoracic ganglion	0.00	0.08
Thoracic muscle	0.00	0.25
Digestive system	0.66	1.34
Total	0.66	3.96

*Total of all tissues assayed equals analysis of whole fly.

ABSORPTION AND TRANSPORT OF DDT

The results of attempts to locate the sites of degradation of DDT in resistant flies pointed to the possibility that DDT may not be as widely and generally transported through the body of insects as one would suspect upon reading the literature on the subject. Läuger *et al.*, (1946) reports the recovery of sufficient DDT from the thoracic ganglia of 50 poisoned flies to kill an additional 5 flies. He also reports the recovery of sufficient DDT from the malpighian tubules and gut of a like number of poisoned flies to kill 30 flies. Work of a similar nature done by Bot (1949) would seem to confirm these studies. The latter demonstrates the presence of DDT in the haemolymph of DDT-poisoned insects by transfusing the blood from such insects into normal insects where typical poisoning symptoms developed. He also reports the presence of DDT in the malpighian tubules of poisoned insects as shown by bioassays of extracts of these structures. All attempts on our part to duplicate the findings of these workers have resulted in failure. In view of the fact that the manipulations involved are exceedingly difficult to master, it should be pointed out that our failure to confirm the results of these workers may have been the result of faulty technique on our part.

It seemed, however, that if DDT is to be found in the internal tissues of poisoned flies to the extent indicated by the studies of Läuger *et al.* and Bot, that a chemical analysis of extracts of these tissues should reveal, in a relative manner, its distribution. Groups of fifty DDT-susceptible flies from which the mouthparts had been removed were treated individually with a dosage of 0.5 μg. of DDT per fly applied in 0.5 mm.^{3} of ethanol to the mesonotum of CO_2 anesthetized flies. This volume of DDT solution will approximately cover the thorax, with little or none flowing on to the adjacent areas of the head and abdomen. The different lots of flies, thus treated, were rinsed with ether after varying intervals following treatment to remove DDT from the body surfaces. The flies were then frozen until they could be dissected and the various tissues and body regions analyzed for DDT.

TABLE IV

RECOVERY OF DDT FROM POOLED TISSUES DISSECTED FROM 50 SUSCEPTIBLE FLIES TREATED TOPICALLY WITH 0.5 μG. DDT PER FLY, AFTER THE REMOVAL OF THEIR MOUTHPARTS

TISSUE ANALYZED*	μG. DDT RECOVERED PER FLY		
	3.5 Hours	9 Hours	24 Hours After Treatment
Entire head	0.00	0.00	0.00
Thoracic cuticle, legs and wings	0.08	0.17	0.21
Thoracic muscles, fore gut and ganglion	0.00	0.00	0.00
Entire abdomen	0.00	0.00	0.00
External body surface	0.30	0.20	0.14
Total recovered	0.38	0.37	0.35

*Total of all tissue assayed equals analysis of whole fly.

The results of this study are presented in Table IV. It is apparent from these data that the amount of DDT applied to the flies is for the most part to be found on the external parts of the body even in flies assayed 24 hours after treatment. This is shown by the high recovery of DDT in the ether rinse of the flies. The most significant point is the fact that DDT is not recoverable from the internal tissues of the flies either as DDT or as a metabolite which will respond to the Schechter test. On the other hand, a substantial quantity of DDT is found in the cuticle-hypoderm of the area where the treatment was applied. In all cases it was impossible to account for all of the total dosage of DDT applied. This loss is not due to errors in extraction or other causes because control experiments performed on similar tissue homogenates treated with known dosages of DDT invariably yield 95 to 99 per cent of theoretical quantities on extraction.

These results make it seem impossible that amounts of DDT reported by Läuger (1946) and Bot (1950) could be present in various

internal tissues as indicated by their bioassay studies of tissue extracts from poisoned flies. Our results support the findings of Roeder and Weiant (1946), Bodenstein (1946), and others, who as a result of physiological experiments believe the effects of DDT may be peripheral in nature. In view of the fact that substantial quantities of the applied dosages were not recoverable as DDT or a known metabolite, the possibility of DDT being converted to a toxic metabolite is not excluded as a possible explanation of the results of Läuger *et al.*, and Bot. Another possibility is that DDT may be interfering with the normal metabolism of cells in the periphery of the insect to cause them to secrete a toxin which is bioassayable in more centrally located tissues and the haemolymph.

TABLE V

RECOVERY OF DDE APPLIED TOPICALLY TO SUSCEPTIBLE AND DDT-RESISTANT HOUSE FLIES; 10 FLIES IN EACH GROUP

TIME IN HOURS	STRAIN OF FLY	μG. DDE APPLIED	μG. DDE RECOVERED PER FLY		
			External	Internal	Total
1.5	Susceptible	10	6.07	2.03	8.10
5.5	"	10	5.45	3.75	9.20
10.0	"	10	4.03	5.66	9.69
1.5	Resistant	10	8.95	0.92	9.87
5.5	"	10	7.50	1.72	9.22
18.0	"	10	4.50	4.95	9.45

ABSORPTION AND METABOLISM OF DDE

It was previously pointed out that susceptible flies treated with DDT and assayed at varying intervals following treatment show a gradual loss of DDT which cannot be accounted for by any known metabolite of DDT. It was thought that this loss might be accounted for if it should be found that susceptible flies readily degrade DDE to an unknown product. A comparison was therefore made between DDT-resistant and susceptible flies in respect to the absorption and fate of DDE applied topically.

The results of this study are shown in Table V. In the case of both strains, DDE was recovered in near theoretical quantities in all instances. Both strains absorb DDE at about the same rate and neither strain is affected in any obvious way at the dosage at which it was applied. It is apparent that the loss of DDT encountered after application to susceptible flies cannot be explained by the assumption that DDE may be a transitory intermediate in the formation of an unknown metabolite.

ABSORPTION AND METABOLISM OF DDA

In order to check the possibility that DDA (bis- (p-chlorophenyl) acetic acid) might be a transitory metabolite in the metabolism of DDT by susceptible flies, groups of 10 flies were treated topically with 10

μg. DDA per fly in 1 mm.³ of ethanol. The flies were rinsed with ether after 18 hours to remove DDA that had not yet been absorbed. It seemed likely that DDA, once absorbed, would be converted to its salt. Therefore, the tissues of one group of flies were acidified with 3 cc. 6N H_2SO_4 to convert all the DDA present to the acid form. The tissues were then extracted as usual with ether. Another group of flies was extracted without first making the tissues acid. The extract of this group thus only contained DDA present in the flies as such.

The results of this experiment are given in Table VI, along with data from a similar test with DDT-resistant flies. Since 95 to 100 per cent of the DDA could be recovered up to 30 hours after treatment when the tissues were acidified before extraction, it is apparent that neither strain can degrade DDA. Low recoveries when the tissues were

TABLE VI

RECOVERY OF DDA APPLIED TOPICALLY TO SUSCEPTIBLE AND RESISTANT HOUSE FLIES; 10 SUSCEPTIBLE FLIES AND 20 RESISTANT FLIES IN EACH GROUP

TIME IN HOURS	STRAIN OF FLY	μG. DDA APPLIED	TREATMENT OF FLIES BEFORE EXTRACTION	μG. DDA RECOVERED PER FLY		
				External	Internal	Total
18	Susceptible	10	Acidified	4.90	4.55	9.45
18	"	10	Not acidified	4.75	1.55	6.30
30	Resistant	5	Acidified	3.38	1.60	4.98
30	"	5	Not acidified	3.38	0.65	4.03

not acidified prior to extraction, indicate that DDA, after being absorbed, is converted to its salt in both strains of flies.

The inability of flies to degrade DDA rules out the possibility that DDA may be an intermediate transitory product in the metabolism of DDT by susceptible flies.

IN VITRO STUDIES ON DDT DEGRADATION

The foregoing studies suggested the desirability of an attempt to degrade DDT by incubation with various tissues of resistant and susceptible flies. Previous attempts to demonstrate the degradation of DDT with the whole fly *brei* of resistant and susceptible flies were erratic, in that occasionally considerable quantities of DDE could be obtained by this method from the *brei* of resistant flies, but the results could not be consistently reproduced. It is worth stating, however, that in all cases where the *brei* of susceptible flies was used, we were able to recover only DDT, and usually in theoretical quantities.

Various tissues of susceptible and resistant flies were dissected from the living flies and placed in 3 cc. of Kreb's ringer solution containing 2% glucose and buffered to a pH of 7.0. An amount of DDT in ethanol solution, approximating that which might be used on an equivalent number of flies, was then added to the Ringer solution. The amount of ethanol introduced was 5 mm.³ per 50 μg. DDT. These tests

were incubated in Warburg respirometers in order to maintain constant agitation and a temperature of 37° C. The results of these preliminary attempts are shown in Table VII.

In several instances, the ethanol solution of DDT was applied directly to the sections of cuticle before immersion in Ringer solution for incubation. By this method no degradation occurred even when resistant fly cuticle was used, and it seems likely that the cells were killed by the ethanol. When the ethanol solution of DDT was added to the Ringer solution, and the DDT thus present as a fine suspension, degradation to

TABLE VII

RECOVERY OF DDT AND DDE FROM FLY TISSUES INCUBATED IN 3 CC. KREB'S RINGER PLUS 2% GLUCOSE, pH 7.0, WITH DDT IN SUSPENSION

SUSCEPTIBLE FLIES	μG. DDT ADDED	RECOVERED		TIME INCUBATED IN HOURS
		μg. DDT	μg. DDE	
10 abdominal cuticles	47	45	0.0	3
5 " "	19	18	0.0	26
5 " "	14*	12.8	0.0	26
RESISTANT FLIES				
10 thoracic cuticles	43	35.4	3.9	4
10 abdominal cuticles	45	42.8	3.5	4
10 " "	46	41.8†	0.8	7
10 " "	46	35.3	2.2	12
10 " "	46	37.7‡	3.5	18
50 " "	52	47.2	3.0	14
50 " "	54	44.7	8.9	17
10 " "	40*	36.5	0.0	12
10 " "	40*	37.5	0.0	12
10 digestive tracts	25	27.0	0.0	12
10 brain and thoracic ganglion	25	25.0	0.0	12
5 puparia contents	49	48.5	0.0	4
50 abdominal cuticles centrifugate (mostly cuticle)	88	89	0.0	20.5
supernatant (cellular disintegrates)	81	78	0.0	20.5

* DDT applied topically to the cuticle before immersion in Ringer.

† Cuticle and Ringer analyzed separately. 19.8 μg. DDT and 0.8 μg. DDE on the cuticle. 22 μg. DDT in Ringer.

‡ Same. 17.2 μg. DDT and 3.5 μg. DDE on cuticle. 20.5 μg. DDT in Ringer.

DDE by the cuticle-hypoderm of resistant flies could be demonstrated. The per cent degraded was in all cases low, only in one instance approaching 20 per cent of the total DDT present. No tissues tested, other than the cuticle-hypoderm of resistant flies, have been found able to degrade DDT *in vitro*. It should, however, not be concluded that other tissues cannot bring about the degradation of DDT, since the optimum conditions for *in vitro* degradation are not by any means known. It is evident, though, that this resistant strain of fly does possess a mechanism in the peripheral region of the cuticle-hypoderm which can degrade DDT to DDE *in vitro*.

METABOLISM OF DDT WHEN APPLIED TO LARVAE

The larval stage of the house fly has been observed to be relatively more resistant to DDT than the adult. Larvae from our susceptible strain taken two days before reaching the normal date of pupation are capable of tolerating 10 μg. of DDT applied as a topical application. Larvae thus treated successfully enter the pupal stage, but rarely emerge therefrom, as adult flies. The same stage of larvae treated with 1 μg. of DDT applied topically will enter the pupal stage and transform to adult flies with the same survival as untreated controls. Larvae from our resistant strain when topically treated with 10 μg. of DDT at this

TABLE VIII

RECOVERY OF DDT AND DDE FROM RESISTANT AND SUSCEPTIBLE LARVAE AFTER TOPICAL APPLICATION OF DDT TWO DAYS BEFORE NORMAL DATE OF PUPATION. TEN LARVAE WERE ASSAYED AT EACH TIME INTERVAL

TIME IN HOURS AFTER TREATMENT	STRAIN OF LARVA	μG. DDT APPLIED	RECOVERED PER LARVA			
			External	Internal		Total*
			μg. DDT	μg. DDT	μg. DDE	μg. DDT
1	Resistant	10	9.90	0.00	0.10	10.00
2	"	"	9.80	0.02	0.30	10.15
3	"	"	9.50	0.01	0.41	9.96
5	"	"	9.45	0.09	0.24	9.80
9.5	"	"	9.25	0.18	0.42	9.89
15	"	"	9.20	0.09	0.82	10.19
20	"	"	8.20	0.43	0.51	9.21
1	Susceptible	10	8.70	0.05	0.00	8.75
2	"	"	8.45	0.10	0.00	8.55
4	"	"	7.10	0.25	0.00	7.35
14	"	"	7.20	0.38	0.00	7.58
26.5	"	"	6.25	0.95	0.00	7.20
1	Susceptible	1	0.89	0.03	0.00	0.92
2	"	"	0.83	0.05	0.00	0.88
3	"	"	0.84	0.08	0.00	0.92
13	"	"	0.43	0.23	0.00	0.66
26	"	"	0.33	0.16	0.00	0.49

* Total recovery based on molecular weight of DDT.

stage successfully enter the pupal stage and emerge as adults with approximately the same percentage survival as untreated larvae. Thus it is apparent that the larvae of the two strains differ in respect to their tolerance to DDT.

Larvae of the two strains of flies were treated in the manner previously described and placed in petri dishes without food until they were extracted and assayed for DDT and its metabolites. The data from this experiment are summarized in Table VIII.

It is at once obvious that tolerance in the case of each strain is not due to the inability of DDT to penetrate the cuticle. The rate of

absorption of DDT by the larvae of both strains is in the same order or greater than that found by Sternburg *et al.* for the adults. There are several obvious differences between the two strains with respect to the metabolism of DDT. First, the resistant strain has the facility to convert DDT to DDE and store it in considerable quantity as such, whereas, not a trace of DDE is to be found in the susceptible larvae. Second, it is possible to account for near theoretical quantities of the DDT applied to resistant larvae 20 hours following treatment, while in the case of susceptible flies, there is a gradual and unaccountable loss of DDT as the time between treatment and extraction is extended. These

TABLE IX

RECOVERY OF DDT AND DDE FROM VARIOUS SOURCES OF RESISTANT ADULTS 6–8 DAYS AFTER TOPICAL TREATMENT OF THE LARVAE WITH 10 μg. DDT PER LARVA. TWENTY INSECTS PER GROUP

MATERIAL ASSAYED	RECOVERED PER INSECT			
	Group I		Group II	
	μg. DDT	μg. DDE	μg. DDT	μg. DDE
Rubbed off in flask	5.05	0.00	4.50	0.00
Empty puparium	3.25	0.00	2.85	0.00
External on adult	0.04	0.08	0.04	0.06
Internal:				
Cuticle	0.00	0.85	0.00	0.49
Brain and thoracic ganglion	0.00	0.00	0.00	0.00
Digestive tract and sex organs	0.00	0.10	0.00	0.03
Thoracic muscles	0.00	0.10	0.00	0.05
Total	8.34	1.13	7.39	0.63
Total based on molecular wt. of DDT	9.59		8.09	

observations suggest that the susceptible strain of larvae possess a mechanism for metabolism of DDT into a product which does not respond to the Schechter test. The fact that resistant larvae do not show a comparable unaccountable loss may be due to their ability to convert DDT to DDE before it reaches a site where it would otherwise be converted to unknown metabolites. Evidence supporting this speculation is provided in Table V, where it was shown that neither strain possessed any marked ability to further degrade DDE in any reasonable interval of time after topical treatment.

Further evidence of the retention of DDE by resistant flies was shown by the following study, in which resistant larvae were treated topically with 10 μg. of DDT per larvae one day prior to pupation and then held until they became adults (6–8 days). Results of the analysis of these adults are shown in Table IX. In view of the number of manipulations

involved in the dissection process, the recovery of 80 to 95 per cent of the original dosage after 6–8 days had elapsed is extremely high, and it seems safe to assume that degradation beyond DDE has not taken place. Again, as in the dissection of topically treated resistant adults, only DDE was recovered from internal tissues of the flies, and the greater part of the DDE recovered appears to have been retained in the cuticle-hypoderm throughout the period of transformation. Since the amount of DDT absorbed was only that which would be absorbed by resistant larvae in approximately 20 hours (Table VIII), it appears that absorption after pupation must have been negligible. This observation led to the study of DDT absorption by puparia.

ABSORPTION AND METABOLISM OF DDT BY THE PUPARIA

In this study newly formed puparia of both strains of flies were given topical dosages of 10 μg. DDT per puparium, and then held 24

TABLE X

RECOVERY OF DDT AND DDE FROM 20 PUPARIA TOPICALLY TREATED WITH 10 μg. DDT PER PUPARIUM

STRAIN	TIME IN HOURS	RECOVERED PER INSECT			
		External μg. DDT	Internal		Total As μg. DDT
			μg. DDT	μg. DDE	
Resistant.....	24	8.80	0.52	0.46	9.83
"	48	8.02	0.26	1.20	9.61
Susceptible.....	24	9.3	0.25	0.00	9.55
"	48	8.45	0.45	0.34	9.28

and 48 hours before analysis for DDT and metabolite. The results obtained are summarized in Table X. No significant difference was found in the rate of absorption of DDT by the two strains. Absorption, however, is slow compared to that of larvae and adults, and even 48 hours after treatment over 80 per cent of the applied dosage can be recovered from the external surfaces. Resistant pupae possess the same facility to degrade absorbed DDT that is present in resistant larvae and adults, and the DDE formed is retained as such. Pupae of susceptible flies treated topically were found after 48 hours to contain some DDE. This is the first time we have recovered DDE from the susceptible strains after topical application of DDT. Since, as shown in the latter part of this paper, larvae of susceptible flies are able to metabolize DDT to DDE by means of the digestive system, it is possible that during the transformations occurring in the pupal stage, a substance normally present only in the digestive tract has been liberated into the body fluids and brought about the partial degradation of the dosage of DDT which was absorbed.

METABOLISM OF DDT WHEN FED TO LARVAE IN CULTURE MEDIA

It was observed that the larvae of resistant and susceptible flies transferred two days before their normal pupation date to larval media containing DDT responded differently to certain dosage levels. It was found that approximately the same percentage of adults could be obtained when resistant fly larvae were put in media containing 1000 p.p.m. as when susceptible larvae were placed in a media containing DDT at the rate of 100 p.p.m. The desirability of comparing the metabolism of DDT in the two strains and through the life stages, larva, pupa, and adult, when exposed to DDT in this manner seemed

TABLE XI

RECOVERY OF DDT AND DDE FROM RESISTANT LARVAE, PUPAE, AND ADULTS PRODUCED FROM LARVAE THAT HAD FED 48 HOURS ON FOOD CONTAINING 1000 P.P.M. OF DDT. 20 LARVAE AND PUPAE PER TEST; 5 ADULTS PER TEST

LIFE STAGE	RECOVERED PER INSECT			
	External		Internal	
	μg. DDT	μg. DDE	μg. DDT	μg. DDE
Larva	0.56	0.16	0.00	11.7
"	0.45	0.05	0.00	13.7
"	0.49	0.13	0.00	16.2
"	0.35	0.13	0.00	16.2
Pupa	0.61	0.21	0.00	17.0
"	0.79	0.34	0.00	18.8
"	0.54	0.27	0.00	19.5
"	0.38	0.27	0.00	18.7
Adult	0.00	1.60	0.00	6.8
Empty puparium	0.86	0.40		

apparent, since the two strains would presumably consume considerable DDT in the process of feeding for 48 hours.

After a 48-hour period of feeding in DDT-treated media, the desired number of larvae were selected from the culture, rinsed to remove surface DDT, and then assayed for DDT and DDE in the usual manner. The larvae remaining in the culture furnished pupae and adults for the other phases of the experiment. The pupae and adults used in these tests were 24 to 48 hours old when they were used. In other respects they were given the same treatment as the larvae.

The data obtained from these experiments are presented in Table XI, where the resistant strain was used, and in Table XII for the susceptible strain. It is assumed that most of the DDT and DDE found in the internal tissues of the larvae gained entry through the intestinal tract. This assumption is supported by the fact that sus-

ceptible larvae treated in this manner were found capable of converting some of the DDT to DDE, whereas topical doses of DDT to this strain do not give rise to DDE when absorbed (Table VIII). If this is true it can be assumed that the intestinal tract of the larva of both strains may affect the conversion. While the two strains of larvae are alike in being able to convert DDT to DDE they differ in the fact that only DDE is to be found in the internal tissues of the resistant strain; whereas, both DDT and DDE are found in the susceptible strain. These data also indicate the greater capacity of the larvae of the resistant strain to degrade DDT. The obvious difference between the amount of DDE found in the adult as compared to that found in the larval and pupal stages of the two strains may possibly be explained by the failure of

TABLE XII

RECOVERY OF DDT AND DDE FROM SUSCEPTIBLE LARVAE, PUPAE, AND ADULTS PRODUCED FROM LARVAE THAT HAD FED 48 HOURS ON FOOD CONTAINING 100 P.P.M. DDT. 25 INSECTS IN EACH LIFE STAGE WERE ANALYZED AS A GROUP

LIFE STAGE	RECOVERED PER INSECT			
	External		Internal	
	μg. DDT	μg. DDE	μg. DDT	μg. DDE
Larva	0.43	0.00	1.88	0.94
Pupa	0.46	0.00	0.99	0.17
Adult	0.00	0.00	0.00	0.10

many individuals to pass through to the adult stage. It is assumed that many of the larvae consumed a dose of DDT which proved fatal to them during pupation, thus those which reached the adult stage may have been only those which consumed the lowest dose in the larval stage.

SUMMARY

Resistant flies, in both larval and adult stages, are able to degrade oral dosages of DDT to the dehydrochlorinated compound DDE. In the adults DDE formed in the digestive tract is liberated into the body tissues and accumulates for the most part in the cuticle-hypoderm. DDT as such does not pass from the digestive tract to other tissues.

Topical application of DDT to resistant larvae, pupae, and adults results in the formation of DDE in the cuticle-hypoderm. When precautions are taken to prevent adult flies from sponging DDT-contaminated surfaces, no DDE or DDT is to be found in any part of the body except the cuticle-hypoderm where detoxification apparently occurs. DDE, once formed, is retained in the body and not metabolized further. Very little is excreted. When resistant larvae are treated topically with DDT, it has been shown that the adults produced by these larvae contain DDE, mainly in the cuticle-hypoderm, and that almost complete

recovery of the original dosage can be obtained, partly from the empty puparia as DDT and the rest from the adults as DDE.

Topically treated adult susceptible flies, with their mouthparts removed to prevent ingestion of DDT, absorb DDT into the cuticle-hypoderm only. No DDT can be recovered from other tissues of the body. The DDT absorbed by the cuticle-hypoderm is partly metabolized to an unidentified substance not responding to the Schechter test. It is possible that this fraction of the dosage, not responding to the color test, may actually be responsible for the toxic action of DDT. Other alternatives are that DDT present in the cuticle-hypoderm causes a toxin to be produced by the cells, that is then carried in the haemolymph to the rest of the body, or that DDT acts directly on the peripheral nervous system without the aid of an intermediate toxin.

Larvae of susceptible flies treated topically give results similar to those found for the adults. When fed DDT, susceptible larvae possess a weak ability to form DDE. This ability to degrade DDT to DDE in the digestive tract is not developed to the extent found in resistant larvae, where degradation is complete even at higher dosage levels. It is evident, though, that the mechanism by which DDT is degraded to DDE is present to a certain extent in the intestinal tract of the susceptible strain of flies.

Neither DDE nor DDA can be metabolized by either strain of fly. Close to theoretical recovery can be obtained 20 hours or more after topical treatment, even though the greater part of the dosage has been absorbed by the insect. DDA that is absorbed is partly converted to its salt and as such is retained by the body. Thus neither of these compounds can be a transitory intermediate in the metabolism of DDT by susceptible flies to an as yet unidentified compound.

Puparia of both strains of flies are relatively impermeable to DDT applied topically in ethanol solution. Over 80 per cent of a 10 μg. dosage of DDT can be recovered 48 hours after application. The small amounts absorbed are converted to DDE by both strains; almost completely by the resistant strain, but only to a small extent by susceptible pupae. Susceptible pupae thus differ from their larvae and adults in this respect, since neither have been found able to form DDE after topical application of DDT.

In vitro studies have been made on various tissues of both strains, and although the optimum conditions are not yet known, it has been shown that the cuticle-hypoderm of resistant flies can degrade DDT to DDE *in vitro*. Other tissues have not yet shown this ability and in the case of susceptible fly cuticle no degradation can be demonstrated.

The data and results listed above lead to the conclusion that the cause of resistance to DDT by certain strains of flies is due to the development of a mechanism by which DDT is detoxified to DDE in the cuticle-hypoderm and in the digestive tract before it has reached a vital site.

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THE NEARCTIC LEAFHOPPERS (HOMOPTERA: CICADELLIDAE), A GENERIC CLASSIFICATION AND CHECK LIST, by PAUL WILSON OMAN. Mem. Ent. Soc. Washington, No. 3. 253 pages, 44 plates. 1949.

This is one of the most outstanding recent contributions to the study of one of the most difficult families of the Homoptera. Dr. Oman is due the deep appreciation of all students of leafhoppers for having made a thoroughgoing taxonomic survey of these insects.

The introduction covers the broader aspects of our knowledge of this family and includes an historical resume, problems of classification, life histories, host plants and food plants, economic importance, leafhopper vectors of plant virus diseases, parasites, distribution, technique, morphological notes, terminology, and bibliographic information.

This is followed by the taxonomic part. There is a key to the subfamilies. The subfamilies are treated according to a standard pattern with descriptions of the subfamilies, keys to the tribes, if necessary, descriptions of the tribes, and keys to the included Nearctic genera. The generic descriptions include complete bibliographic references to original descriptions and type designations and is followed by an alphabetic list of species with bibliographic citations to original descriptions, general distribution, and synonymy.

The specific synonymy seems to have been especially well handled.

The genus *Aphrodes* Curtis lists six species, five of which are Palearctic species that have been introduced into our fauna; but in this genus not less than thirty-six synonyms are listed. Yet in handling such a complex only two minor errors are apparent: *bicincta* Curtis is cataloged as a synonym of *costata* Panzer but is not listed among the synonyms of that species, and *testudo* Curtis is cataloged as a synonym of *costata* Panzer but is listed as a synonym of *albifrons* Linnaeus.

Another fundamental taxonomic problem fully recognized by the author is the proper definition and association of the subfamilies of the leafhoppers. While he believes that the group treated in this work as a single family with eighteen subfamilies will eventually be divided into several families, he does not believe that our knowledge has progressed far enough to enable one to do this successfully at this time.

No review of this contribution to knowledge would be complete without reference to the forty-four fine plates which include figures of the dorsal and facial and sometimes the lateral view of the heads and of the fore and hind wings of most of the genera.

There are also illustrations of the nymphs of some of the more common genera and a few illustrations of the genitalia. Having held for many years the view that the male genitalia could furnish more reliable generic characters than most of the structures now used to define genera, I cannot help but regret that this feature was not greatly expanded.

This contribution will find a place on the work table of every student of Nearctic leafhoppers. It could well serve as a model for the students of other orders of insects not only from our fauna but from all regions of the world.

Z. P. METCALF.

NEUERE PROBLEME DER ABSTAMMUNGSLEHRE, by BERNARD RENSCH. vii+407 pp., 102 fig. Ferdinand Enke Verlag, Stuttgart. 1947.

This interesting, well-documented, synthetic treatise, prepared by a foremost student of evolution, is of unique interest since, because of war-time limitations on communications it was written without access to the recent English-language treatises in the same general field including principally "Systematics and the Origin of Species" by Ernst Mayr (Columbia University Press, 1942), "Evolution, the New Synthesis" by Julian Huxley (Harpers, 1942), and "Tempo and Mode in Evolution" by G. G. Simpson (1945). All of these treat aspects of evolution discussed by Doctor Rensch. This affords an unusual opportunity to compare conclusions reached independently and based, in part at least, on different fundamental materials. Although there are some instances of disagreement, the reader cannot resist the impression of a rather remarkable concurrence in most aspects of basic philosophy. No reader can fail to be impressed favorably with the author's admirable capacity to employ a huge variety of materials and sources of information, an appreciable portion of which is entomological. Emphasis is primarily on trans-specific processes but as a basic introduction there is initially a concentrated review of the processes and factors involved in specific and intraspecific evolution with attention to the kinds and causes of mutations, random nature of mutations, pleiotropy and interaction of genes, fate of genes in respect to fluctuations in populations, and the mechanisms of natural selection. Naturally-occurring races (subspecies) are classified as (1) historic, (2) geographic, (3) ecologic, (4) sexual, (5) genetic, and (6) hybrid. Geographic races are believed to be the primary precursors of new species although ecologically, physiologically (sexually), and genetically isolating processes are usually also involved. Processes involved in speciation are regarded as the same as those in subspeciation and Goldschmidt's suggestion of chromosome mutations (in contrast to gene mutations) as basic to speciation is rejected.

Most of the book is devoted to a consideration of the adequacy of random mutation and natural selection as basic explanations for the processes involved in trans-specific evolution, and the many manifestations of these processes in terms of orthoselection, parallel selection, and allometric effects, to mention only a few. The development of most evolutionary series shows a *specialization phase* the extent of which is, to a certain extent, a function of the variety and relative saturation of biocoenoses. There are very interesting considerations of the basic phenomena involved in the development of certain structures at the expense of others (reconstruction). Allometric relations, pleiotropy, interaction of genes, and selection in terms of optimal utilization of energy and synthetic materials in ontogeny are to be considered. *Parallel structures*, such as similar wing patterns in different families of Lepidoptera, are to be explained basically in terms of the occurrence of homologous genes with orthoselection and/or the favorable selection of mimics of distasteful species. There is an interesting section (pp. 80–111) dealing with the absolute rate of evolution. Among the Arthropoda evolution has been more rapid in the terrestrial groups as is the case also among the Vertebrata. Present species may have evolved 100,000 to 500,000 years ago whereas recognizable geographic races have developed during recent historic times. Rate of evolution is a function of environment in terms of several ramifications, conditions for selection being probably the most important. "Predisposition" of existing structural plans makes the assumption of increased mutation rates during periods of rapid evolution unnecessary. However, increased mutation rate is offered as a possible explanation of the so-called explosive developmental periods such as that of insects during the Carbonaceous and Permian; development of new biotopes and other environmental factors are doubtless involved also. These are only the briefest and most fragmentary glimpses of a rich array of ideas and examples which can be appreciated only by careful scrutiny of the entire book. Of particular interest to entomologists should be the section (pp. 240–260) on the action of evolution on different stages in life cycles. The section (pp. 316–325) entitled "Die Evolution des Lebendinger" is perhaps the weakest. One raises the question of the advisability of an attempt to discuss such a complex subject, which should involve many biochemical physiocochemical and thermodynamic considerations, in nine pages. The concluding sections (pp. 326–373) are profoundly philosophical and probe deeply into the very fundamental causal precepts of biology and natural science.—DONALD S. FARNER.

OBITUARY NOTICE

PERCY NICHOL ANNAND. On the morning of March 29, the Bureau of Entomology and Plant Quarantine of the U. S. Department of Agriculture, for the second successive time lost its active head through death. No doubt weakened by serious operations earlier in life, and further affected by the heavy burdens of his official work, Dr. P. N. Annand died from a heart condition at the age of 51, thereby depriving the Bureau of a leadership that should have been available to it for years to come.

According to official sources of information, Dr. Annand was born at Telluride, Colo., in 1898, was graduated from Colorado Agricultural College in 1920, worked for the Great Western Sugar Company in 1920–1921, was an assistant at Stanford University in 1921–2, where he received an M.A. degree in 1922, was head of the Biology Department of the San Mateo, California, Junior College from 1922 to 1929, during which period he studied also at Stanford University and received a Ph.D. degree in 1928. His first association with the Bureau came in 1929, when he joined its sugar beet leafhopper investigation project, working in the western U. S. for three years. Brought to Washington as assistant leader of the Truck Crop Insect Division in 1932, his obvious capabilities led him, in the brief period of the following nine years, through positions as head of the Cereal and Forage Insect Division, Special Research Assistant to the Chief of Bureau and Assistant Chief of Bureau for Research to Chief of Bureau in 1941.

While it is not the proper function of this notice to discuss or to evaluate Dr. Annand's services to economic entomology—such a review surely will be presented in the more appropriate vehicle which is available—brief mention of some aspects of his relation to this field seems permissible. Thus, in a day when opinion is sometimes voiced that insect taxonomy represents only a pioneering, and presumably primitive, phase of economic entomology, it is perhaps worth noting that Dr. Annand acquired his basic training in entomological research from a painstaking, critical study of the classification of the North American species of one group of insects (Adelginae). It may be noted, too, that Dr. Annand's leadership of the Bureau coincided with the greatly augmented entomological activity of the war period, with the outstanding development of a whole new group of insecticides and new methods of treatment for insect control, and with the need to meet new and critical problems in economic entomology, stemming in large measure from the war and its aftermath; problems, for example, so varied as those arising from the great increase in the rapidity and diversity of world transportation that followed the war, and from the marked increase in post-war international cooperation through United Nations and other agencies.

To all of the widely varied activities of the Bureau, old or new, Dr. Annand made the contributions consistent with his position as Bureau leader, displaying throughout his remarkable talent to absorb the smallest details of any problem, to correlate these details, to develop from them and to present a simple, clear, picture of the problem, from basic causal factors to probable ultimate solution. The success of his leadership is attested from many sources, but perhaps the strongest and certainly the most human evidence of this is to be found in the extraordinary outpouring of contributions from Bureau personnel and others when the time came to pay final floral tribute to the man and his work.

—HAROLD MORRISON.

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